WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 889-898. Research Article ISSN 2277– 7105

IN - VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF *VIOLA SERPENS* AND *MORUS NIGRA* AGAINST PATHOGENS ISOLATED FROM PATIENTS SUFFERING FROM JAUNDICE

Anu Kumar*[1], Manisha Kumari[1], Rita Singh Mazumdar[3] and Tejpal Dhewa[2]

[1]Department of Life sciences, Himachal Institute of Life Sciences Paonta Sahib, H.P (INDIA).

[2]Department of Microbiology, Bhaskaracharya College of Applied Sciences, University of Delhi, (INDIA).

[3]Department of Biotechnology, Sharda University, Greater Noida, U.P (INDIA).

Article Received on
16 Nov 2014,

Revised on 11 Dec 2014,
Accepted on 05 Jan 2015

***Correspondence for Author**
Anu Kumar
Department of Life sciences, Himachal Institute of Life Sciences Paonta Sahib, H.P (INDIA).
anubiotech88@gmail.com

ABSTRACT

In the present scenario there is an urgent need to discover new antimicrobial agents for therapeutic use, as resistance to current drugs increase in severity and extent on the passage of time. Our research work was focused on the identification of natural products from leaves extracts of *Morus nigra* and *Viola serpens* in order to decipher the antibacterial activity against bacteria causing jaundice disease. Natural products based drugs are known as potential pharmacological agents. The Phytochemical screening of the plants studied showed the presence of Flavonoids, Terpenoids, reducing sugars, amino acids and tannins. In-vitro antibacterial activity was evaluated against strains isolated from patients suffering from jaundice i.e. *Staphylococcus aureus, Pseudomonas aeruginosas, Salmonella typhi, Escherichia coli* and *Klebsiella pneumonia.* Comparative study of Ethanolic extract of *Viola serpens & Morus nigra*with standard antibiotics showed that Ethanolic extract of *Viola serpens* showed maximum antibacterial activity. Concerning the zone of inhibition, the values were merely higher for those caused by the plant extracts as compared to different antibiotics.

KEYWORDS: Medicinal plants, Antibacterial activity and Phytochemical, Antibiogram.

INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different parts of the country. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavorings and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases.[1] It is a fact that the 25% of all medical prescriptions are based on substances derived from plants or plant-derived synthetic analogues.[2] In fact, according to the World Health Organisation, approximately 25% of modern drugs used in the United States have been derived from plants. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.

MATERIAL AND METHODS

Collection of plant material

The plant material used was the dried leaves of *Viola serpens* and *Morus nigra* Which were collected from forest region of Paonta sahib and Which were identified by Botanical Survey of India Dehradun.

Extraction of plant material

Ethanol extractions were carried out using Soxhlet extractor. Powdered dried leaves (100 grams) were extracted with 200 ml of the solvent. The extracts were filtered using Whattman filter paper no. 1 The filtered extracts were stored in air tight dark bottles at room temperature for antimicrobial activity.[3]

Phyto - chemical screening

Alkaloid: 5g of each sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 h. After filtration, the extracts were concentrated on a water bath to ¼ of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was collected, washed with

dilute ammonium hydroxide and then filtered. The residue obtained is the alkaloid, and was dried and weighed.[4]

Saponin: 1 ml of the filtrate with 2 ml of distilled water, shaken vigorously and then allowed to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 9 to 11 minutes indicates the presence of saponins.

Flavonoids: A portion of the powdered plant samples were separately heated with 10ml of ethyl acetate in a water bath for 3min. The mixtures were filtered and 4ml of each filtrate were shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids.[4]

Tannins

0.5g of each powered samples were boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour.[5]

Glycoside

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.[6]

Terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform.Then concentrated solution of sulphuric acid was added slowly and redish violet color was observed for terpenoid.[6]

Reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Amino acids

Add 2-5 drops of ninhydrin solution to 1 ml of test solution or sample preparation. Mix and keep for 5 min in boiling water bath and observe the development of a pink, purple or violet-blue colour. Imino acids like proline and hydrxyproline give a yellow coloured complex.

Antibacterial activity of *Morus nigra& Viola serpens* leaves extracts

Biochemical characterization

The various biochemical tests were carried out for identification of isolates acooording to procedure given in Cappuccino and Sherman Microbiology A Laboratory Manul 7th edition.

Antibacterial activity of *Morus nigra& Viola serpens* leaves extracts

Bacterial strains

The bacterial strains were isolated from the serum sample of patients suffering from Jaundice i.e., *E.coli, Staphyalococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and S.typhi.*

Antibacterial activity test

Preparation of Inoculums

Using a straight wire touch 5-10 well isolated colonies of particular microorganism against which antimicrobial activity to be tested. Inoculate on the nutrient broth medium. Incubate at 35-37°C for 4 to 6 hour.

Medium

Mueller Hinton Broth and Mueller Hinton Agar are used for the testing of aerobic and facultative anaerobe bacteria. This media has minimal inhibitory effect on antibacterial agents. Mueller Hinton Agar No.2. (HiMedia Laboratory Ltd, Mumbai).

Agar well diffusion method

Anti bacterial activity was carried out using agar well diffusion method for all the seven microbial cultures using Muller Hinton agar medium. Kirby Bauer technique.[7]

Antibiogram pattern of recovered isolates

The antibiotics disc against the bacterial species sp., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhi* isolated from the serum samples from patients suffering from jaundice were studied.

Disc Diffusion method

The disc diffusion method was used. In this, 0.1 ml of inoculums of test organisms was spread on Muller Hinton Agar media. 6 discs were placed gently with the help of forceps. Then the plates were incubated for 24 hrs. at 37^0C. Observed the plates for zone of inhibition in mm and measured the zone by using scale.

RESULTS & DISCUSSION

Phytochemical screening of plant materials

Qualitative analysis of ethanolic leaves extracts of both plants (*M.nigra & V.serpens*) showed the presence of flavonoids, Tannins, Terpenoids and Reducing sugars. Polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits, which include lowering of blood lipids.[8] Qualitative analysis carried out on each plant extract showed the presence of phytochemical constituents and the results are summarized in Table1.

Table 1: Phytochemical constituents of *Viola serpens* & *Morus nigra.*

Phytochemicals	*M. nigra*	*V. serpens*
Alkaloids	-	-
Saponins	-	-
Tannins	+	-
Amino acids	-	+
Terpenoids	+	-
Reducing sugars	-	+
Glycosides	-	-
Flavonoids	-	+

Antibacterial Activity of Ethanolic extract of *Morus nigra & Viola serpens*

Preparation of test microorganisms

The samples were collected in sterile container from the civil hospital of Paonta Sahib. The bacterial isolates were recovered from serum samples by enrichment culture technique. Portion of sample measuring 1 ml were added in 5 ml of nutrient broth on the very same day of collection, incubated at 37^0 C in incubator for 24 hours. Enriched samples were then streaked on 2 different media, Mannitiol Salt agar (MSA), Pseudomonas Isolation agar (PIA) and Nutrient agar (NA). The various morphological characteristics of recovered isolates viz., colony morphological (colour, shape, arrangement and gram staining) were studied. The various biochemical tests were carried out for identification of isolates.

Determination of antibacterial activity

The present study was designed to identify the antibacterial activity of ethanolic extracts of *Morus nigra* and *Viola serpens* which was also compared with six standard antibiotics against clinical isolates from the serum samples of patients suffering from jaundice disease. The antibacterial activity was monitored using agar-well diffusion and agar disc diffusion method; activity was determined by noting the zones of inhibition around the wells or discs.[9] Table below showed that the percentage of isolates was as follows.

Table 2: Percentage of isolates.

Isolates	Total number of isolates recovered from 15 samples	Total percentage (%)
Gram negative		
E.coli	9	60%
P.aeruginosa	8	53.3%
K.pneumoniae	6	40%
S.typhi	7	46.6%
Gram positive		
S.aureus	8	53.3%

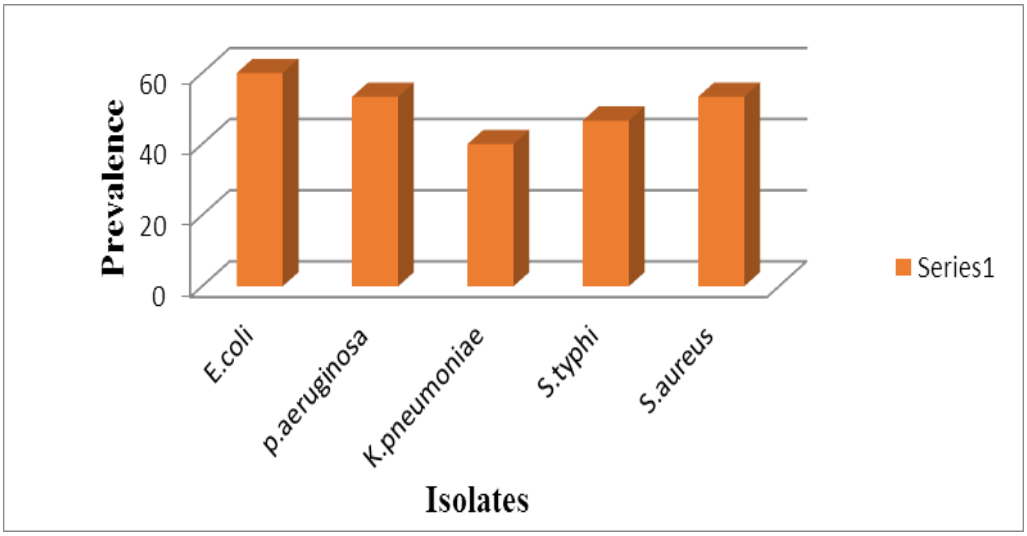

Fig 1: Isolates recovered from serum samples of patients suffering from Jaundice.

The present study showed the prevalence of above 5 recovered isolates from different 15 serum samples of patients suffering from jaundice. This indicates that these bacteria are prominent in causing jaundice among different individuals. Among these isolates, *E.coli* is most prominent in samples taken from jaundice patients.

Table 3: Comparative study of Antibacterial activity of ethanolic extract of *Morus nigra* with different antibiotics.

Plant/ Antibiotics used	Zone of inhibition (mm)				
	E.coli	***S. aureus***	***P.aeruginosa***	***K.pneumo***	***S.typhi***
Morus nigra (ethanolic)	10mm	16mm	14mm	12mm	14mm
Ofloxacin (OF^{5})	Nil	3mm	Nil	6 mm	3 mm
Gentamicin (G^{10})	10 mm	Nil	Nil	4mm	8 mm
Ciprofloxacin (CIP^{10})	12 mm	10 mm	Nil	14 mm	6 mm
Chloramphenicol (C^{10})	2 mm	12 mm	6 mm	8 mm	6 mm
Carbenicillin (CB^{100})	Nil	12 mm	Nil	10 mm	10 mm
Kanamycin (K^{5})	14 mm	3 mm	Nil	6 mm	3 mm

Table 4: Comparative study of Antibacterial activity of ethanolic extract of *Viola serpens* with different antibiotics.

Plants/Antibiotics used	Zone of inhibition (mm)				
	E.coli	***S. aureus***	***P.aeruginosa***	***K.pneumoniae***	***S.typhi***
Viola serpens (ethanolic)	16 mm	19 mm	17 mm	12 mm	22 mm
Ofloxacin (OF^5)	Nil	3 mm	Nil	6 mm	3 mm
Gentamicin (G^{10})	10 mm	Nil	Nil	4 mm	8 mm
Ciprofloxacin (CIP^{10})	12 mm	10 mm	Nil	14 mm	6 mm
Chloramphenicol (C^{10})	2 mm	12 mm	6 mm	8 mm	6 mm
Carbenicillin (CB^{100})	Nil	12 mm	Nil	10 mm	10 mm
Kanamycin (K^5)	14 mm	3 mm	Nil	6 mm	3 mm

Above Tables showing the comparative study of Ethanolic extract of *Viola serpens* & *Morus nigra* with standard antibiotics.It has been found that Ethanolic extract of *Viola serpens* showed maximum antibacterial activity, while Ethanolic extract of *Morus nigra* comparatively showed less antibacterial activity. Concerning the zone of inhibition, the values were merely higher for those caused by the plant extracts as compared to different antibiotics. ZOI values ranged from 0 - 14 mm for antibiotics while they were much higher ranging from 10 - 22 mm for the plant extracts.

Antibacterial activity of Plant extracts against recovered isolates

Control

Fig 2: *E. coli*

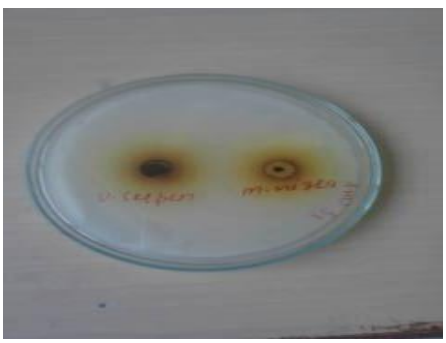

Fig 3: *S. aureus*

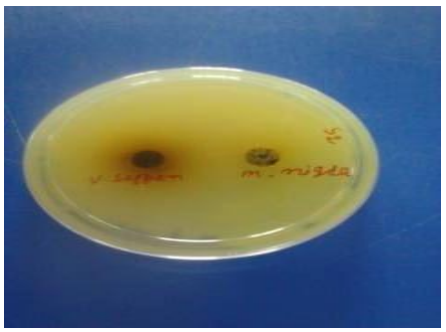

Fig: 4 *P. aeruginosa*

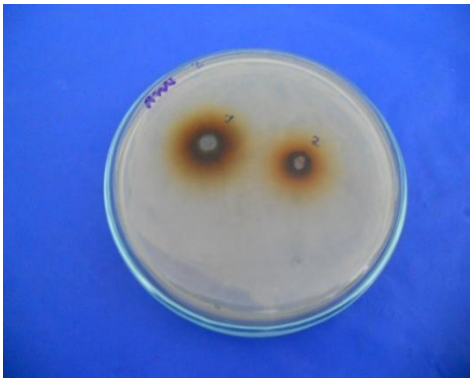

Fig: 5 *K. pneumonia*

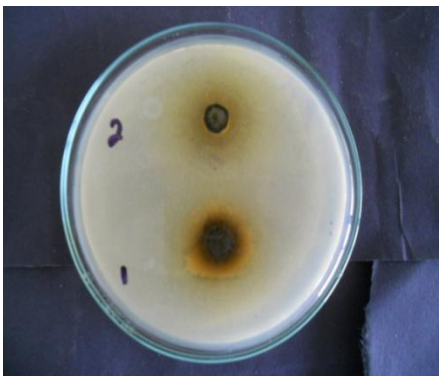

Fig: 5 *S. typhi*

Antibiogramm pattern of recovered isolates

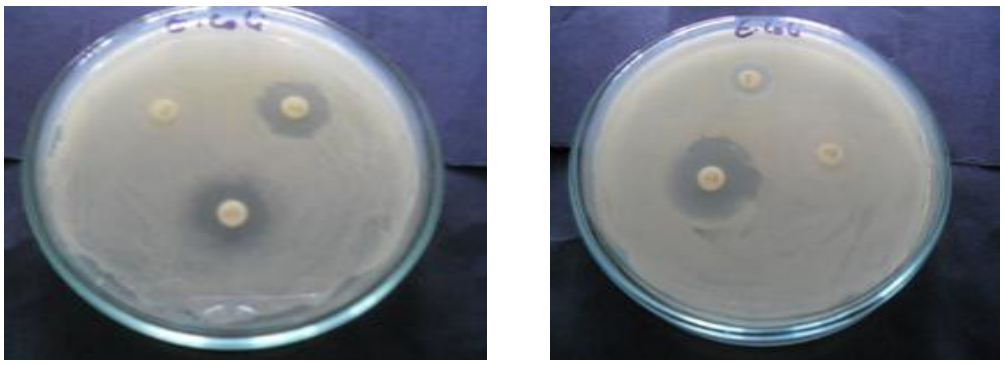

Fig: 6 *E. coli*

Fig: 7 *S. aureus*

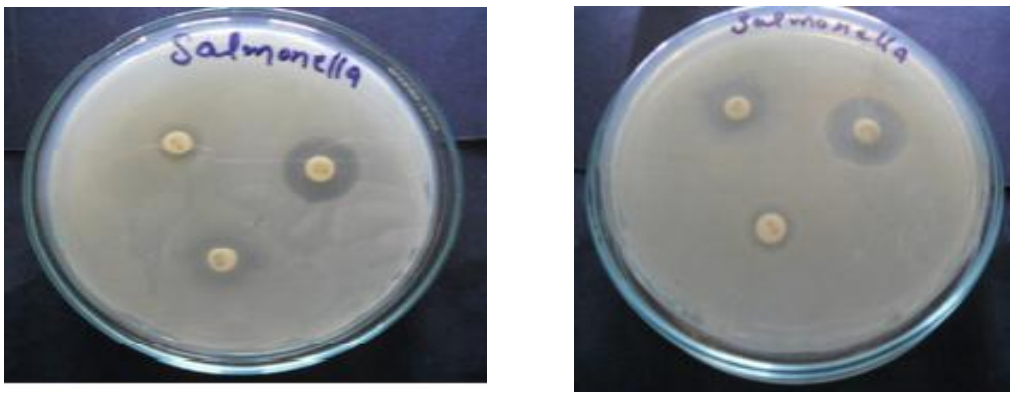

Fig: 8 *S. typhi*

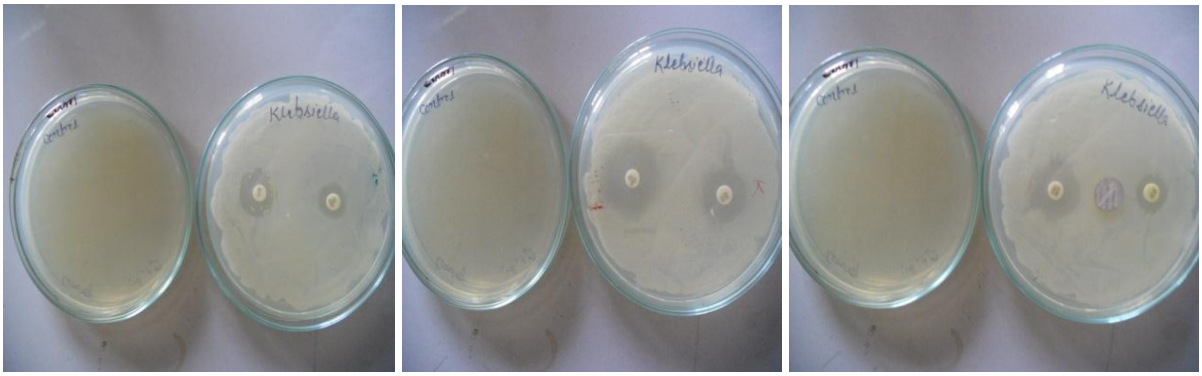

Fig: 9 *K. pneumonia*

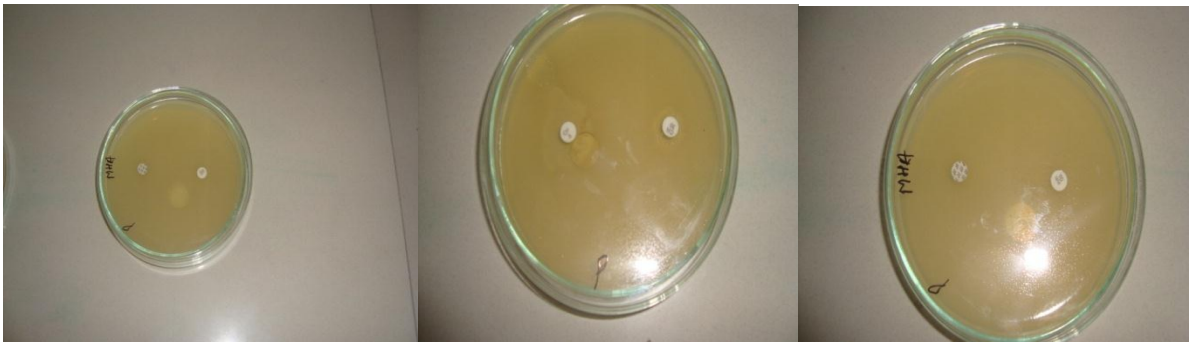

Fig: 10 *P. aeruginosa*

The present study has revealed the importance of natural products to control antibiotic resistant bacteria, which have been a threat to human health. Pathogenic bacteria are responsible for many human infections and if the growth of infectious bacteria is not checked, it may lead to serious health problems. Antibiotics are being used to check such bacterial infections. Plant extracts are also very important tool against such infections. Andallu *et al*., 2001 reported blood serum glucose reduction by mulberry which was used in the old Chinese herbal medicine. It also has the ability to reduce blood cholesterol and lipid levels, effective against arterial plaques, diuretic and expectorant.[10] As the bacterial strains are becoming resistant to the antibiotics already in use, there is a continuous need for newer antimicrobial agents preferably of plant origin to avoid side effects. The dry powder of mulberry leaf was reported to have antioxidative activity in streptozotocin diabetic rats.[11]

CONCLUSION

From the present study it has been concluded that ethanolic extract of *Viola serpens* showed a broad spectrum of antibacterial activity against bacteria isolated from the serum samples of patients suffering from jaundice.These medicinal plants can be used in popular medicine as remedies for many infectious diseases.

ACKNOWLEDGEMENTS

We are thankful to Chairmen and Director of Himachal Institute of Dental Sciences Paonta Sahib, Distt. Sirmour (H.P) for providing the necessary facilities for the above experiments.

REFRENCES

1. Stary F and Hans S, The National guides to medical herbs and plants. Tiger Books. Int. Plc. UK (1998).
2. Sara V, Franca T, Gelsomina F, Traditional uses of medicinal Plants in Valvestino (Italy), J. Ethnopharmacol, 2009; 121: 106–116.
3. Chukwuka K S, The antimicrobial activities of some medicinal plants on *Escherichia coli* as an agent of diarrhoea in livestock, Pelagia research library, Adv. Appl. Sci. Res, 2011; 2(4): 37-48.
4. Harborne, J B. Phytochemical methods, London. Chapman and Hall, Ltd., 1973; 49-188.
5. Trease G E, Evans W C, Pharma cognsy. 11th Edn. Brailliar Tiridel Can. Macmillan Publishers,1989.
6. Siddiqui A A, Ali. Practical Pharmaceutical chemistry, CBS Publishers and Distributors, New Delhi, Ist ed., 1997; 126-131.
7. Bauer A W. Antibiotic susceptibility testing by standardise single disc or cup method, Amer. J. Clin. Path, 1966; 45(4): 493- 496.
8. Manach C, Scalbert A, Morand C, Polyphenols: food sources and bioavailability. Am J Clin Nutr., 2004; 79: 727–47.
9. Gul N, Mujahid TY, Ahmed S. Isolation, identification and antibiotic resistance profile of indigenous bacterial isolates from urinary tract infection patients. Pak. J. Bio. Soc., 2004; 7(1): 2055-2060.
10. Jang M H, Kim H, Shin M C, Lim B V, Lee T H, Jung S B, Kim C J, Kim E H, Administration of folium Mori extract decreases nitric oxide synthase expression in the hypothalamus of rats. Jpn. J. Pharmacol, 2002; 90(2): 189-192.
11. Andallu B and Varadacharyulu Nch. Antioxidant role of mulberry (*Morus indica L*. cv. *Anantha*) leaves in streptozotoc in diabetics rats. Clin Chim Acta, 2004; 215: 217-218.