Unique Research Journal of Chemistry
Available online: www.ujconline.net
Research Article

# ANTIMICROBIAL ACTIVITY OF ANDROGRAPHIS *PANICULATA NEES* AND RECUPERATIVE EFFECT ON MIXING WITH ANTIBIOTICS

Shobana M[1], Maheshwari R[1*], Syed Muzammil M[2*]

[1]PG & Research Department of Biochemistry, K.M.G College of Arts & Science, Gudiyattam Tamilnadu-India
[2]PG & Research Department of Biochemistry, Islamiah College (Autonomous) Vaniyambadi, Tamilnadu-India.

Received: 11-07-2013; Revised: 18-08-2013; Accepted: 17-09-2013

*Corresponding Author: M.Syed Muzammil,
Assistant Professor & Research Supervisor, Department of Biochemistry, Email:syed_bio2004@yahoo.co.in

## ABSTRACT

The present investigation is part of continuing programme related to the phytochemical screening of Andrographis *Paniculata Nees* (Kalmegh) and effect of antibiotics mixing with the Andrographis *Paniculata Nees* (herbal extracts) their enhanced potential on microbes. In the world the local plants used in Ancient Indian Medicine, Ayurveda, Siddha and Yunani, in several countries including India several plant species are administered orally to control the various diseases. Some of these plants have been pharmacologically provided to be of some value and may be a popular remedy for the treatment of disease.

From the very beginning of human existence, man has familiarized himself with medicinal plants and used them in a variety of ways throughout the ages. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action. This relationship has grown between plants and man, and many plants came to be used as drugs. The growth of knowledge to cure disease continues at an accelerating pace, and number of new plant-derived drugs increase likewise. Herbal medicine is currently experiencing a revival in Western society along with other complementary therapies such as traditional Chinese Medicines, Osteopathy and Homeopathy. In this context, the present study is the first milestone with particular emphasis on the application of Andrographis *Paniculata Nees* (kalmegh) the medicinal plant and their effect in mixing with antibiotics for their better formulation and controlling the various diseases in near future.

**Keywords:** Andrographis *Paniculata Nees,* E.*coli,* B.*subtilis, S.aureus,* S.*epidermis,* Methanol, Roxythromycin, Levofloxacin, Cefixime.

## INTRODUCTION

The use of plants as medicines represent the biggest human use of the natural world plants provides the predominant ingredients of medicines in most medical traditions. There are vast number of medicinal plant on earth, the number and percentage for countries and regions vary greatly. Based on positive therapeutic results, herbal medicines are gaining popularity worldwide for human wellbeing and healthcare. One major hurdle that might impair their potential future are "medicine of choice" is the lack of standardization. The breakthrough in chemical marker and identification promise herbal medicines a challenging era. For further classification, the treatment may be compared using poly herbal syrup, with the various antibiotics mixing and their effectiveness. Thus, it is concluded that the efficacy of mixing up of antibiotics and its bioactive compounds may have more significant effect on the various diseases and antimicrobial activities.

In view of the above considerations the present study was designed to investigate the protective effect and their antibiotics mixing of Moreover, antimicrobial activity content were seen.

Andrographis *Paniculata Nees* (Kalmegh) belongs to the family of Acanthaceae and is popular worldwide with the name of "King of Bitters" in English. It is an annual herbaceous plant which is widely cultivated in Southern Asia, India, China and some parts of Europe. Some of the common names are the Kalmegh, Kalafath, Kan-jang, Alui, Charita, Cherota, Chiraita, Cheretta, Kariyat, Green Chiretta, Halviva, Kreat, Sinta, Rice bitters, Sambilata, Sambiloto, Kraut, Andrographidis. The leaves and roots have traditionally been used over the centuries in Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. Andrographis *paniculata* (Kalmegh) has an important place in the Indian Pharmacopoeia and is one of the most widely used plants in ayurvedic formulations[1]. The whole plant has variety of therapeutic values. It has immunosuppressive properties and is useful in treatment of wounds, ulcers, leprosy, sore throat, and

hypertension[2]. Panchang (stem, leaves, flowers, root and seeds) of the plant is being used in various formulation of Indian system of medicine for the treatment of fever, malaria and sore throat[3]. Andrographis *paniculata* has been used in the treatment of some skin infections in India by folkloric medicine practitioners. It is considered beneficial to the skin and is used both internally and externally for this purpose[4]. The plant is also reported effective against diarhoea[5] and it is commonly used to prevent and treat common cold[6]. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia[7]. The plant contains a number of diterpenoids. However, the major bitter constituent is andrographolide, which is diterpene lactone. Diterpenoids and flavonoids are the main chemical constituents of Andrographis *paniculata* which are believed to be responsible for the most biological activities of this plant[8]. Two flavonoids, identified as 5, 7, 2', 3'-tetramethoxyflavonone and *5-hydroxy-7, 2',3'*-trimethoxyflavone, as well as several other flavonoids were obtained from the whole plant[9]. The bitter principle andrographolide was isolated in pure form by Goiter[10]. Andrographolide is also attributed with some other activities like liver protection[9] anticancer activity[10] anti-diabetic activity[11] and anti-malarial activity[11]. The plant extract exhibits anti typhoid, antifungal, antiviral[12] and anti-pyretic[13] activities. It is also reported to possess anti-inflammatory and anti snake venom properties[14]. Recent research has thrown light on cultivation of this plant on large scale because of its high medicinal value. Hence, the present investigation was taken up with an objective to evaluate the antimicrobial potential against the microorganisms.

## MATERIALS AND METHODS

### CHEMICALS

All the fine chemicals were purchased from Sigma chemical co., USA. All other chemicals used were of good quality and analytical grade.

### PHYTOCHEMICAL ANALYSIS

Qualitative analysis of phytonutrients was done for methanolic extract[15,16].

**Alkaloid:**

Meyer's test (potassium mercuric iodide-1.36g of mercuric chloride was dissolved in 60ml of distilled water and 5g of KI in 10ml of water. The 2 solution were mixed and dilute to 100ml with distilled water). To 1mlof acidic aqueous solution of samples few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloid.

**Test for proteins**

To the test solution the Biuret Reagent is added. The blue reagent turns violet in the presence of proteins.

**Test for sugars**

The substance was mixed with equal volume of Fehling's A and B solutions, heated in water bath. Formation of red colour is the indication of the presence of sugar.

**Tannins:**

In a test containing 5ml of extracts a few drops of 1% solution of lead acetate was added. A yellow or red precipitate was formed including the presence of tannins (Lead acetate test).

**Test for coumarins**

To the test sample 10% of sodium hydroxide and chloroform were added. Formation of yellow colour indicates the presence of Coumerin.

**Test for gum**

To the substance, add few ml of water and shake well. Formation of swells or adhesives indicates the presence of gum.

**Saponins:**

In a test tube containing about 5ml of an extracts a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3minutes. A honey comb like froth was formed and it showed the presence of saponins.

**Resin:**

To 2ml of extract 5-10ml of acetate anhydride was added, dissolved by gently heating code and then 0.5ml of sulphuric acid was added. Bright purple colour was produced. It indicates the presence of resins**.**

**Flavonoids:**

In a test tube containing 0.5ml of extract of the sample 5-10 drops of dilute HCl and small piece of Zn or Mg were added and the solution was boiled for few minutes. In the presence of flavonoides, reddish pink or dirty brown colour was produced.

**Glycosides:**

A small amount of extract was dissolved in 1ml of water and then aqueous sodium hydroxide solution was added. Formation of yellow color indicates the presence of glycosides.

**Steroids:**

To 2ml of extract of sample 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red colour was produced in the chloroform layer.

**Phenols:**

To 1ml of extract 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green indicates the presence of phenols (Ferric chloride test).

**Terpenoids:**

2ml of concentrated sulphuric acid was added to 1mg of extract and observed for reddish brown colour.

**Cardiac Glycoside:**

Total 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid a brown ring obtained at the interface indicates the presence of de-oxysugar characteristics of cardenolides.

**Test for Quinones**

To the test substance, sodium hydroxide was added. Blue green or red colour indicates the presence of Quinone.

**Triterpenoids:**

10mg of the extract acetic acid was added following the addition of concentrated sulphuric acid. Formation of reddish violet colour indicates the presence of triterpenoids.

### PREPARATION OF PLANT EXTRACT

500 mg of the herbal powder samples were separately taken in conical flasks along with 10ml of Methanol. The mixture was then allowed to stand overnight and after that the extract was filtered out. This procedure was repeated thrice. The solids

obtained were reconstituted such that the final concentration set was 100mg/ml till complete extraction was ensured.
Preparation of the combinations of the three antibiotics such as Roxythromycin, Levofloxacin and Cefixime were dissolved in double distilled water. Various mixtures were made in combination with the Andrographis *Paniculata Nees* (Kalmegh) extract. The Andrographis *Paniculata Nees* leaves extract was abbreviated as APMLE.

**Table 1: Phytochemical screening of the aqueous extract of Andrographis *Paniculata Nees***

| Name of the Tests | Aqueous Extract of Andrographis *paniculata Nees* |
|---|---|
| For Carbohydrates | |
| Molisch's Test | + |
| Benedict's Test | + |
| For Amino Acids | |
| Ninhydrin Test | - |
| For Alkaloids | |
| Dragendroff's Test | - |
| Mayer's Test | - |
| For Sterols | |
| Libermann reaction | + |
| For Triterpenoids | |
| Salkowski Test | - |
| For Phenolics & Tannins | |
| Lead acetate Test | + |
| For Flavonoids Glycosides | |
| Shinoda Test | + |
| Alkaline Test | + |
| For Saponins Test | |
| Foam Test | - |

**'+' – Positive, '-' – Negative**

**ANTIMICROBIAL ACTIVITY**

The microorganisms were collected from the PG & Research Department of Microbiology K.M.G College of Arts & Science, Gudiyattam-Tamilnadu. The antimicrobial activity was evaluated using disc and streak plate method.

**TEST MICROBES USED**

i) Staphylococcus *aureus*
ii) Staphylococcus *epidermis*
iii) Escherichia *coli*
iv) Bacillus *subtilis*

**PREPARATION OF THE MEDIUM**

2.8gms of nutrient agar was weighed correctly and dissolved in 100ml of sterile distilled water. pH was adjusted to 7.2 and was autoclaved at 121°C for 15 minutes. 20ml of molten agar was poured in to the sterile petri plate and allowed to solidify.

**SUSCEPTIBILITY TESTS**

**DISC METHOD**

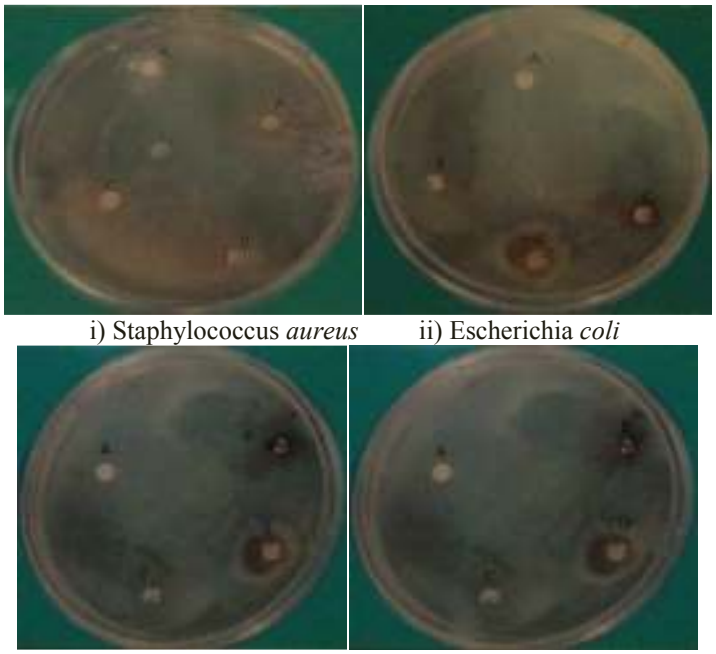
The Whatmann No.1 filter paper discs were made and the plate method is the most commonly used technique for determining susceptibility of micro organisms to know the concentration of antibiotics. Whatmann No.1 paper disc impregnated with combination extraction agents were placed upon the surface of pre inoculated plate. The plates were incubated at 37°C for 24 hours. Susceptibility of effectiveness was observed by the diameter of the inhibition zone around the Disc. Organisms which grew up to the edge of the disc were resistant.

**STREAK PLATE METHOD**

Petri plate containing Nutrient Agar was seeded with the combination mixture and allowed to solidify. Overnight grown cultures were than taken one by one and streaked on the plate. A control plate continuing only nutrient agar devoid of antibiotic mixture was simultaneously streaked and incubated. The growth was observed against the control plate.

**TABLE 2: SAMPLE PREPARATIONS**

| Sample Name | Combinations |
|---|---|
| A1 | APMLE (500mg) + Methanol (10ml) |
| A2 | APMLE + Roxythromycin |
| A3 | APMLE + Cefixime |
| A4 | APMLE + Levofloxacin |



i) Staphylococcus *aureus* ii) Escherichia *coli*

iii) Staphylococcus *epidermis* iv) Bacillus *subtilis*

**Figure 1: Effect of mixing antibiotics and their antimicrobial activity**

**TABLE 3: DISC METHOD**

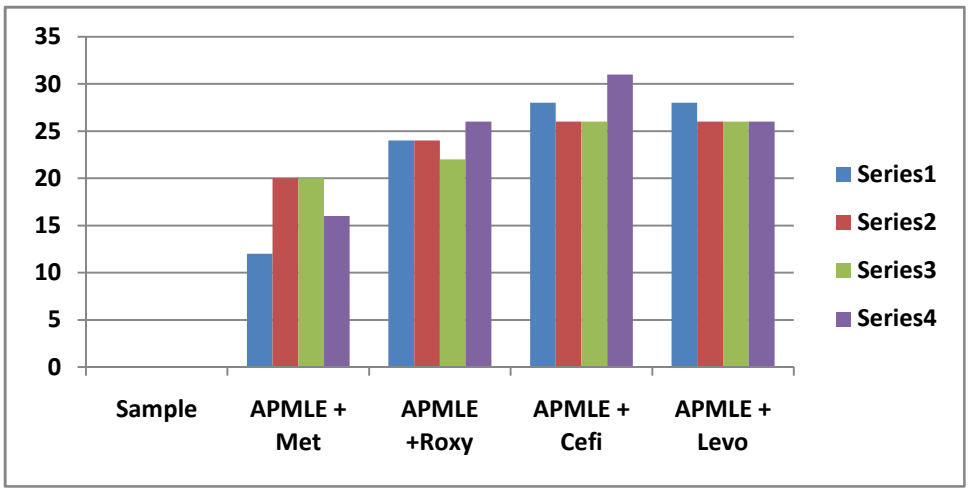
| Sample Name | Zone (mm) | I | Zone (mm) | I | Zone (mm) | I | Zone (mm) | I |
|---|---|---|---|---|---|---|---|---|
| APMLE + Methanol | 12 | I | 20 | S | 20 | S | 16 | S |
| APMLE+Roxythromcyin | 24 | S | 24 | S | 22 | S | 26 | S |
| APMLE + Cefixime | 28 | S | 26 | S | 26 | S | 31 | S |
| APMLE + Levofloxacin | 28 | S | 26 | S | 26 | S | 26 | S |

**I: Interpretation, Zone: - <8 R – Resistant 8 to 16 I- Intermediate >16 S- Sensitive.**

**TABLE 4: STREAK PLATE METHOD**

| Sample Name | Microbes | 100mg/ml | 250mg/ml | 500mg/ml | 750mg/ml | 1g/ml |
|---|---|---|---|---|---|---|
| APMLE + Methanol | S.*aureus* | ++ | +++ | ++ | +++ | +++ |
| APMLE + Methanol | S.*epidemis* | ++ | ++ | ++ | ++ | +++ |
| APMLE + Methanol | E.*coli* | + | ++ | + | + | ++ |
| APMLE + Methanol | B.*subtilis* | + | ++ | + | ++ | ++ |
| APMLE+ Roxythromycin | S.*aureus* | +++ | +++ | +++ | +++ | +++ |
| APMLE+ Roxythromycin | S.*epidemis* | +++ | +++ | +++ | +++ | +++ |
| APMLE+ Roxythromycin | E.*coli* | +++ | +++ | +++ | +++ | +++ |
| APMLE+ Roxythromycin | B.*subtilis* | +++ | +++ | +++ | +++ | +++ |
| APMLE + Cefixime | S.*aureus* | + | + | ++ | + | +++ |
| APMLE + Cefixime | S.*epidemis* | + | + | + | + | +++ |
| APMLE + Cefixime | E.*coli* | + | + | + | ++ | +++ |
| APMLE + Cefixime | B.*subtilis* | + | + | +++ | ++ | +++ |
| APMLE + Levofloxacin | S.*aureus* | +++ | ++ | ++ | ++ | ++ |
| APMLE + Levofloxacin | S.*epidemis* | + | ++ | +++ | ++ | ++ |
| APMLE + Levofloxacin | E.*coli* | ++ | ++ | ++ | +++ | ++ |
| APMLE + Levofloxacin | B.*subtilis* | ++ | ++ | +++ | +++ | ++ |

**+ Less inhibition, ++ Medium, +++ Complete, ---- No inhibition.**




**GRAPH 1: Effect of mixing antibiotics and their antimicrobial activity**

## CONCLUSION

The present study summaries plant-derived incorporated antibiotics which are widely prescribed world wise and are considered natural, safe, and beneficial. Interest has revived recently in the investigation of medicinal plants to identify novel active phytochemicals that might lead to drug development as more potent antibiotics, antimalarials, anticancer agents antihyperglycemic agents etc derived from research on plants.

The plants gives slow effect on the microbes but when it is combined with the drugs mixture gives high zone of inhibition. Although the plant extract gives high effect on Staphylococcus *epidermis* and Escherichia *coli* the effect become higher when combine. Table-3 Shows the different concentrations of extract and combination. It helps to find out the perfect concentration of drugs which gives highest inhibition of pathogens.

Antimicrobial effect of the extract may be attributed to the phytochemical constituents such as sterols and terpenes, polyphenols, flavonoids, tannins saponins, and alkaloids. These compounds would be having strong activity due to the phytochemicals and could explain the antimicrobial activity of the plant. However, more detailed phytochemical analysis is required to isolate and characterize each active compound which is responsible for the antibacterial activity and exact mechanisms of action of this activity.

The allopathic forms of antimicrobials give adverse effects to the human system and to the metabolism. So, therefore we need an antimicrobial which has lower side effects and high effectiveness. The combination of high amount of herbal extracts and low amount of drugs will give new path in medicinal world. It is a new concept to combine herbal and Allopathy drugs to be known as herbo-allopathy combinations.

## ACKNOWLEDGEMENTS

We are grateful to the faculty members of both the colleges, PG & Research Department of Biochemistry & Microbiology, Principal, Islamiah College and K.M.G College, Principal, Secretary & Correspondent, Islamiah College, General Secretary and VMES for their encouragement and support in carrying out the work.

## REFERENCES

1. Hooker J.D, Flora of British India, 1885; Vol. IV, L. Reeve & Co. Ltd. Ashford, Kent.
2. Puri A, R. Saxena, R. P. Saxena, K. C. Saxena, V. Srivastava and J. S. Tandon, Immunostimulant Agents from Andrographis *Paniculata*, J. Nat. Prod., 1996; 56 (7), 995-999.
3. Chopra R.N, S. L. Nayar and I. C. Chopra, Glossary of Indian Medicinal Plants. 1956; 1st Ed. Publication and Information, New Delhi.
4. Jain S.K, Dictionary of Indian Folk Medicine and Ethnobotany- A Reference Manual of Man-Plant Relationships, Ethnic Groups and Ethno Botanists in India, 1991; Deep Publication, New Delhi, India.
5. Saxena S, D. C. Jain, R. S. Bhakuni and R. P. Sharma, Chemistry and Pharmacology of Andrographis Species, Indian Drugs, 1998; (35):458-467.
6. Caceras, D.D, J. L. Hancke, R. L. A. Burgos and J. K. Wick Man, Prevention of Common Cold with Andrographis Paniculata Dried Extract, A Pilot Double Blind Trial, Phytomed., 1997; (4): 101-104.
7. Handa S.S and A. Sharma, Hepatoprotective Activity of Andrographolide from Andrographis *Paniculata* Against Carbon Tetrachloride, Indian J. Medicinal Res., 1990; (92): 276-283.
8. Sharma, P.V, Dravyaguna vigyana, Kushmanda, 1st Chapter, Part-II, 2003; Chaukhambaha Bharati Academy, Varanasi, Reprint, pp-17.
9. Tang, W and G. Eisenbrand, Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine, 1992; Springer Verlag, Berlin, 97-103.
10. Rao K.Y, G. Vimalamma, C. V. Rao and Y. M. Tzeng, Flavonoids and Rographolides from

Andrographis *Paniculata,* Phytochem., 2004; 65(16):2317-2321.
11. Gorter M.K, the Bitter Constituent of Andrographis *Paniculata* Nees. Rec. Trav. Chim., 1911; (30): 151-160.
12. Saraswat S, P. K. S. Visen, G. K. Patnaik and B. N. Dhawan, Effect of and Rographolide Against Galactosamine Induced Hepatotoxicity. Fitoterapia, 1995; (66):415- 420.
13. Kumar R.A, K. Sridevi, N. V. Kumar, S. Nanduri and S. Rajagopal, Anticancer and Immunostimulatory Compounds from Andrographis *Paniculata*, J. Ethnopharma., 2004; 92(2-3):291-295.
14. Lal J, H. C. Tripathi and S. K. Tandon, Antidiabetic Activity of Andrographolide Indian J. Pharmacol., 1986; (18):58-61.
15. Rahman N.N, T. Furuta, S. Kojima, K. Takane and M. A. Mohd, Antimalarial Activity of Extracts of Malaysian Medicinal Plants, J. Ethnopharmacol., 1999; (64):249-254.
16. Chang R.S, L. Ding, C. G. O. Hen, Q. C. Pan, K. L Zhao and K. M. Snith Dehydrographolide Succinic Acid Monoester as an Inhibitor Against the Human Immunodeficiency Virus, Proceedings of Society of Experimental Biology and Medicine, 1991; (197):59-66.
17. Madav S, H. C. Tripathi, S. K. Tandan and S. Mishra, Analgesic, Antipyretic and Antiulcerogenic Effect of Andrographolide, India J. Pharmaceut. Sci., 1995; (57):121-125.
18. Sofowora A. Screening plants for Bioactive Agents. In: Medicinal plants and Tradditonal Medicine in Africa. (2nd Edn.), Spectrum Books Ltd, Sonshine House, Ibadan, Nigeria, 1993, 134-156.
19. Trease GE and Evans WC: Pharmacognosy. (15th Edn.), Sawnders Publishers, London, 2002, 42-44.
20. Barry, A.L. *et al*., Macrolide resistant to bacteria, 1997; The American Journal of Medicine, (40).
21. Bingen, E. *et al*., Resistant to macrolides in staphylococcus, American Society for Microbiology, 2000; (44).
22. Chetty, R. S. and Ravikumar, P.R, Chemistry of Ayurvedic crud drugs, Botanical Journal, 1987; 6: (26).
23. Dhar, M.L., Dhar, M.M. and Dhawab, B.N., Screening of Indian medicinal plants for biological activity, 1986; American Botanical Journal, 232.
24. Eltenza, J.M. *et al.,* Levofloxacin a review of its antibacterial activity, pharmacokineties and therapeutic efficacy Drug,1994; Philosophical Analysis in latin America, 677-700.
25. Godkar, P.B, Textbook of Medicinal Laboratory Technology, Bhalani Publication House, 2nd Ed, 203; 489-500.
26. Jain, P.C. and Ahmed, S., Chemical Examination of Asparagus racemosus, Bulletin of Medico Ethnobotanical research, 1991; 12(3-4):157.
27. John, B.M., Antibacterial Antibiotic, Wilson and Gisvold's Organic Medicinal and Pharmaceutical Chemistry, 2004; 11th Ed. 49.
28. Kokate, C.K., Purohit A.P. and Gokhale S.B., Pharmacognosy by C.K.kokate, Nirali Prakashan, 2007; (181-183):2001.
29. Langtry, H.D. and Lamb, H.M., Levofloxicn its use in infections of the respiratory tracts, Martindale-The Complete Drug Reference, 1993; 33rd Ed.219.
30. Licata *et al*., Comparison of postantibiotic and Staphylococcus aureus, Staphylococcus epidermis, American Society for Microbiology, 1997; 41.
31. Morrissey, G.J., The bacterial activity of Levofloxacin against bacteria, J Antimicrob Chemother, 1997; (39):719-23.
32. Majumdar, A., Home remedies in Ayurveda, Amar Granth Publications, Delhi, 2002; 390.
33. Mukharjee, K.L., Medicinal Laboratory Technology, McGraw-Hill Education (India) Pvt. Ltd., 2002; (2): 600-604.
34. Neu, H.C., Invitro activity of new broad spectrum cephalosporin, Cefixime, Pediatric Infect Dis J, 1987; (6):954-7.
35. Patel, R.J., Patel, K.R., Experimental Microbiology, Aditya publication, 2000; (2):72-96.
36. Ross, I.A., Medicinal plants of the world, Totova, NJ; Humana Press, 2000; (2):321.
37. Souweine, B. *et al*., Hepatitis with Roxythroycin therapy, DICP Ann Pharmacotherapy 1991 ;( 25):1137.
38. Stone, J., Clinical isolates in –vitro activity of cefixime, J. Antimicrobe Chemother, 1989; 23:221-8.
39. Walters, K.co., The Review of Natural Products by facts and comparisons, St.Louis, MO, USA, Recent Progress in Medicinal Plants,2000; (6).
40. Williamson, E.M., Major herbs of Ayurveda, Churchil Livingstone Publications, 2002; 40.
41. Yasuda, H. *et al.*, Interaction of antimicrobial agents, Biological Research Laboratory, Sankyo Co.Ltd, Tokyo, Japan,3, 1993.

**Source of support: Nil, Conflict of interest: None Declared**