The Bioscan 8(2): 717-721, 2013 (Supplement on Medicinal Plants)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

STUDIES ON ANTIBACTERIAL EFFECTS OF BARK, SEED AND CALLUS EXTRACTS OF *HOLARRHENA ANTIDYSENTERICA* WALL.

SUCHITRA MAHATO*, ANITA MEHTA[1] AND SOMA ROY[2]
Department of Biotechnology, Ranchi Women's College, Ranchi - 834 008 Jharkhand, INDIA
[1]Department of Botany, Ranchi Women's College, Ranchi - 834 008 Jharkhand, INDIA
[2]Department of Biotechnology, Ranchi Women's College, Ranchi - 834 008 Jharkhand, INDIA
e-mail: suchi75tra@gmail.com

KEYWORDS
Holarrhena antidysenterica
Staphylococcus aureus
Salmonella typhimurium
Escherca coli

Received on :
11.12.2012

Accepted on :
22.04.2013

***Corresponding author**

ABSTRACT
A procedure has been outlined for callus formation and antibacterial effects of bark, seed and callus extracts of an important medicinal shrub *Holarrhena antidysenterica* WALL. Explants used for callus formation were mainly seeds and both *in vivo* and *in vitro* grown plant parts like stem, nodal parts, roots and apical shoots. A friable type of callus was obtained when these explants were cultured on Murashige and Skoog (MS) culture medium containing different concentrations of 2,4-D separately and in combinations of KN. During present studies methanolic extracts of bark, seed and callus were made and tested for their antimicrobial activities against *Staphylococcus aureus*, *Salmonella typhimurium* and *Eschercia coli*. Results showed that all three different types of extracts of *Holarrhena antidysenterica* possess nearly similar potential for antibacterial activity against these pathogenic bacteria.

INTRODUCTION

Plants have been used for important medicinal constituents in indigenous medical systems since ancient times. A large proportion of the drugs used in modern medicine are either directly isolated from plants or synthetically modified from a lead compound of natural origin. In plants as a result of metabolic processes many different types of organic compounds or metabolites are produced. Among different plant derived secondary metabolites alkaloids proved to be the most important group of compounds that showed wide range of antimicrobial activity (Sarkar *et al*., 1991; Hossain *et al*.,1993; Raman *et al*., 1997) and since long time it has been established fact that plants are safer alternative sources of antimicrobials (Pretorious and Watt., 2001; Sharif and Banik., 2006). The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dadsena *et al*., 2013; Dandapat *et al*., 2013; Kullu *et al*., 2013; Kumar *et al*., 2013; Kumar *et al*., 2013a; Tabassum *et al*., 2013; Toppo *et al*., 2013; Sahu *et al*., 2013).

Holarrhena antidysenterica WALL. Belongs to family Apocynaceae, a deciduous shrubby 9-10 meter high tree found throughout tropical India, Burma, Srilanka, Pakistan, Nepal and Africa and flowers in the months of May-July. Seeds are linear or oblong concave with a long coma, are light brown in colour and shows epigeal germination. This tree is popular for its numerous medicinal properties and seeds and bark of this tree have been used in Ayurveda since long time. The stem bark which is commonly known as "kurchi" in the Indian subcontinent and as 'conessi bark' in Europe is used in traditional ayurvedic medicine to treat dysentery, especially amoebic dysentery (Bhutani, 1984). Bark of *Holarrhena antidsenterica* is used in Ayurveda as an anti-microbial, anti inflammatory and analgesics (Kirtikar *et al*., 1994; Warrrier *et al*., 1994; Sharma *et al*., 2004). Other useful parts used as medicine are root and leaf. The bark and the roots have been found to be an excellent remedy for both acute and chronic dysentery especially in cases where there is excessive blood with mucus and colic pain associated with stools (Ghosh, 1984). In addition the plant has been reported to possess antihelminthic, appetizing, antidiarrhoel and astringent properties (Chopra *et al*., 1982). These properties are due to the presence of steroidal alkaloids conaine and aminopregnane types, the principal one being conessine. *Holarrhena antidysenterica* is also a rich source of other steroidal alkaloids such as kurchine, kurchimine, conessidine, holarrimine, conessidine, conkurchicine and regholarrhimine (Radt, 1965). *Holarrhena antidysenterica* has been reported to be used as an immunodulating agent (Atal *et al*., 1986), larval growth inhibitor (Thappa *et al*., 1989) and against malaria and vaginities (Hager Handbuch, 1976).

Among 1000 alkaloids isolated from different members of Apocynaceae family, around 30 alkaloids have been isolated

from bark of *Holarrhena antidysenterica* having highest percentage of conessine, a steroidal alkaloid.

The systematic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic bacteria.

The accumulation of phytochemicals in the plant cell cultures has been studied for not more than thirty years and generated knowledge had helped in realization of using cell cultures for production of desired phytochemicals (Evans *et al*., 1986, Purohit and Mathur, 1999, Castello *et al*., 2002). The antimicrobial activity of Apocynaceae is well documented in the literature (Reddy, 2010; Amole and Ilori, 2010; Sharif *et al*., 2006; Suffredini *et al*., 2002; Patil and Ghosh, (2010). Objective of our present investigation is to generate callus tissue and also to evaluate the antimicrobial effects of callus tissue, seed and bark extracts of *Holarrena antidysenterica*.

MATERIALS AND MATHODS

Plant Material

The fresh matured seeds and bark of *Holarrhena antidysenterica* were collected from Charhi colliery forest of Hazaribagh (Jharkhand) after the identification work done by the authentic sources (University taxonomists and local vaidyas). Seeds collected from forest were also sown in garden for plant formation. Seeds as well as different plant parts – stem, node and roots of field grown as well as *in vitro* grown plantlets were used for explants. *In vivo* explants were washed thoroughly in tap water and treated with cetavelon (1:100) for 5 minutes. This was followed by washing with running tap water and then by distilled water. Surface sterilization was carried under aseptic condition in laminar air flow cabinet and treated with 0.2% $HgCl_2$ for 2-3 minutes then rinsed thrice thoroughly with sterile distilled water. Small segments of 1.0 – 1.5cm length of i*n vitro* grown explants were excised from one month old axenic plants and made ready for inoculation.

Culture medium and condition

The basal medium used was MS medium (Murashige and Skoog, 1962) supplemented with 100mgl $^{-1}$(w/v) myo-inositol and 3% (w/v) sucrose and different sets of growth regulators in different concentrations and combinations. Callus initiation, induction and proliferation medium was supplemented with 2,4-D(0.5 – 5.0 mgL^{-1}), BAP(1.0 – 3.0 mgL^{-1}) separately and 2,4-D (2.5 and 1.5 mgL^{-1}) in combination with different concentration of KN (0.5 and 1.0mgL^{-1}); BAP(1.0 – 3.0mgL^{-1}); NAA (0.5mgL^{-1}) and IAA(0.5mgL^{-1}). Callus formed in this medium was white and friable and was subcultured in 5-6 wks intervals on the same medium. The pH of all types of media was adjusted to 5.8 before the addition of 0.8% agar (BDH, India). All chemicals used were of analytical grade (Sigma and Merck). The culture vials containing media were autoclaved at 121ºC and 104 kPa for 20 minutes were kept in a growth cabinet at 25 ± 1ºC, under 16h photoperiod by cool white fluorescent tubes (Philips India) and with 60-65% relative humidity.

Methodology for antibacterial effect

Preparation of plant extracts

Thoroughly washed dried bark and seeds of *Holarrhena antidysenterica* were dried in shade for 15 days and then powdered with the help of waring blender. Calli derived from seed, *in vitro* grown stem, node and root were collected and dried in an oven at 45 ± 1ºC for 48-60h. Dried calli were homogenized to a fine powder and stored in airtight bottles. 10gm of calli powder were mixed with 100mL of Methanol. 25gm coarse powder of bark and seeds were weighed and extracted with 250mL of Methanol. The extract was collected after filtration by using Whatman No. 1 filter paper and then evaporated below 40ºC in water bath (Parekh, J. *et al*, 2005; Mungole, A. J. *et al*, 2008). A sticky material was obtained as final product. 50 mg of crude plant extract (sticky material) was mixed with 50mL of methanol. Thus a stock solution containing 50mg/ 50mL was prepared which is considered as 100% concentrated extract. In this way 75%, 50% and 25% concentrated extract were prepared (Barriada-Pereira, M., *et al*., 2003).

Test microorganisms

In vitro antibacterial studies were carried out against two gram-positive (*Staphylococcus aureus, Salmonella typhimurium*) and Gram-negative (*Escherchia coli*) bacteria. The bacteria were obtained from Dept. of Microbiology, Rajendra Institute of Medical Sciences Ranchi. The bacterial nutrient medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs (121ºC) for 15 minutes. All bacterial species were incubated for 36 hrs at 37 ± 2ºC. After 36h incubation, the growing bacteria were used for further tests.

Antimicrobial activity test

The antimicrobial susceptibility testing was done by using the agar well diffusion method to detect the presence of antibacterial activities of the samples (Perez *et al*., 1990; Harborne, 1998). Microbial growth was determined by measuring the diameter of zones of inhibition measured in mm and the results were recorded.

RESULTS AND DISCUSSION

2, 4-D was the best auxin for inducing as well as for proliferating the callus from seeds during present work. On the other hand, a high concentration of 2, 4-D was found to be toxic as it produced low amounts of callus. The callus appeared white in colour, friable in texture and lacked the potentiality of regeneration. Other explants like stem root and nodal parts were also suitable for callus induction (Graph.1).

The nature of callus, its growth rate, texture as well as colour depend on the constitution of culture media as well as combinations and concentrations of various growth regulators. After every 4-6 weeks the callus was subcultured on the same medium where it grew indefinitely (Fig. 2) and in this way in the present investigation callus was maintained upto four subcultures. In few cultures globular shaped embryoids were observed on the surface of subcultured callus onto the same medium (Fig. 3).

The crude methanolic bark, seed and callus extract obtained from *Holarrhena antidysenterica* were submitted to an antibacterial screening using the agar well method against various pathogenic bacteria. In this study significant zone of inhibition (in mm) was observed on three bacteria (*Staphylococcus aureus*, *Salmonella typhimurium* and

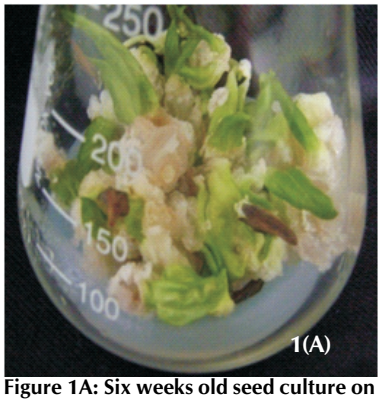


Figure 1A: Six weeks old seed culture on MS +2, 4-D (2.5 mgL^{-1}) showing cotyledonary leaves and callus

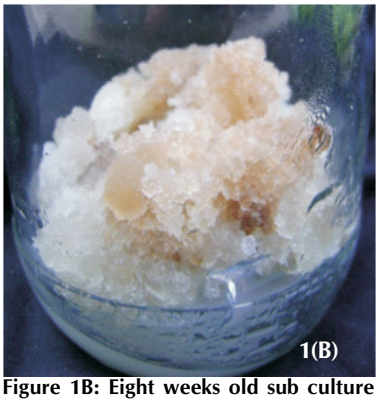
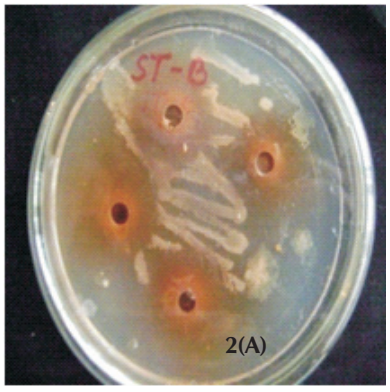
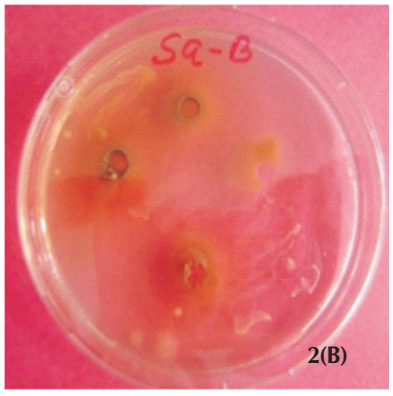
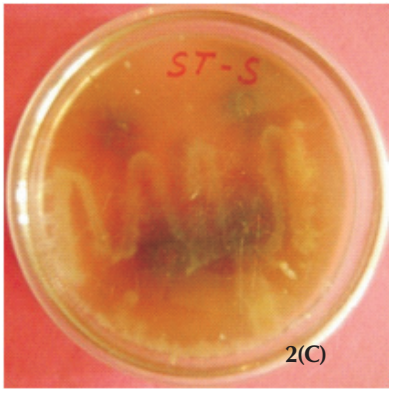


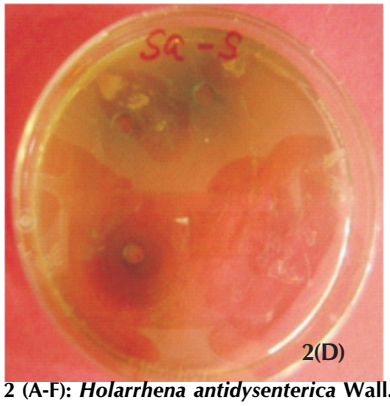
Figure 1B: Eight weeks old sub culture of one and half month seed calli on MS +2, 4-D (2.5mgL^{-1}) showing profused growth of white callus

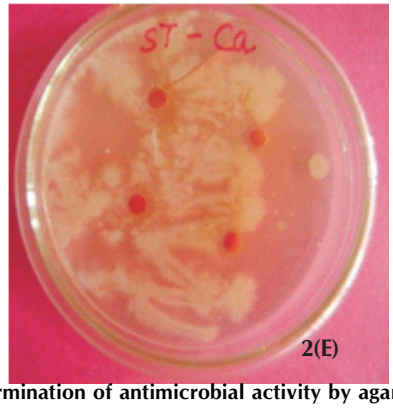
Figure 1C: Six weeks old sub culture of one and half month old seed calli on MS+2, 4-D (2.5 mgL^{-1}) showing globular shaped embryoids on callus surface

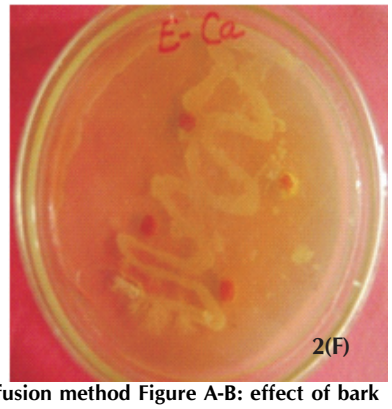








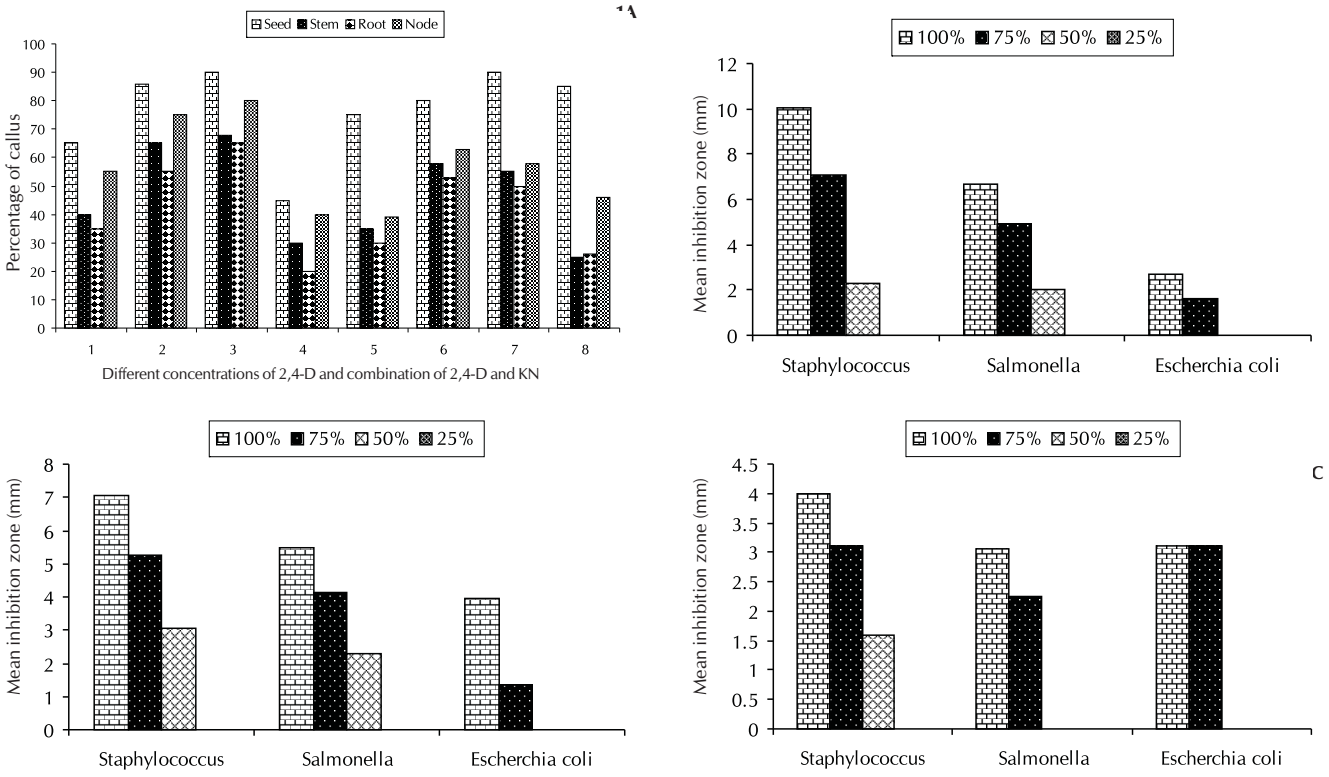




2 (A-F): *Holarrhena antidysenterica* Wall. Determination of antimicrobial activity by agar well diffusion method Figure A-B: effect of bark extract on *Staphylococcus* and *Salmonella* streak culture plate.C-D: effect of seed extract on *Staphylococcus* and *Salmonella* streak culture plate. E-F: effect of callus extract on *Staphylococcus* and *E. coli* streak culture plate

Escherchia coli). During present work about 10.05 mm inhibition zone was observed in bark extract with 100% concentration, showing highest antibacterial activity against *Staphylococcus* (Fig. 2A) whereas in case of *Salmonella and E.* coli it was only 6.65mm and 2.7mm respectively. *Holarrhena antidysenterica* seed extract with 100% concentration also showed antibacterial activity against *Staphylococcus* with inhibition zone of about 7.05mm; *Salmonella* with 5.50mm and *E. coli* with 3.95mm. Callus extracts with 100% concentration showed 4 mm inhibitory zone against *Staphylococcus* and its least activity was observed in *E. coli* with 3.1mm inhibition zone even in 100% concentration.

This is the first report on comparative studies on different types of extracts of *Holarrhena antidysenterica*. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable resistance to bacteria and *Staphylococcus*, the gram positive bacteria was the most susceptible one. Various workers have already shown that gram positive bacteria are more susceptible towards plants extracts as compared to gram negative bacteria (Lin *et al*., 1999; Parekh and Chanda, 2006). In addition microorganisms show variable sensitivity to chemical substances to different resistance levels between different strains of bacteria (Cetin and Gurler, 1989).



Graph 1: Column graph showing percentage of callus formation from seeds and in *vitro* grown different explants of *Holarrhena antidysenterica*. Graph - 2 (A, B, C): Column graph showing antibacterial activity of bark, seed and callus extracts of *Holarrhena antidysenterica*

1 = 2,4-D (0.5); 2 = 2,4-D (1.5); 3 = 2,4-D (2.5); 4 = 2,4-D (5.0); 5 = 2,4-D (1.5) + KN(5.0); 6 = 2,4-D (1.5) + KN(1.0); 7 = 2,4-D(2.5) + KN(2.5) + KN(0.5); 8 = 2,4-D(2.5) + KN(1.0)

Previously antibacterial activity of bark extract of *Holarrhena antidysenterica* was studied (Ballal *et al.*, 2001; Raman *et al.*, 2004; Chakraborty and Brantner, 1999) and investigated alkaloids present in the methanolic extract of bark, a good source of antibiotics. Plant based antimicrobial compounds have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Results obtained in the present study revealed that three types of extracts of *Holarrhena antidysenterica* possess potential antibacterial activity against *Staphylococcus*, *Salmonella* and *E. coli*. Till now it has not been proven whether this effect is due to a single alkaloid or due to the mixture of alkaloids present in the stem bark of *Holarrhena antidysenterica*. Moreover, it has been already demonstrated that conessine is the main alkaloid present in the mixture (Panda *et al.*, 1991). These results supported the ethno medicinal claim that *Holarrhena antidysenterica* contains effective bioactive compounds mainly alkaloids against bacteria causing stomach ailments and three types of extracts of this plant may serve as a valuable source of compounds with therapeutic potential.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. Manju Sinha, Director, Vocational Courses for providing laboratory facilities in Department of Biotechnology, Ranchi women's college, Ranchi University, Ranchi.

REFERENCES

Amole, O. O. and Ilori, O. O. 2010. Antimicrobial activity of the aqueous and ethanolic extracts of the stem bark of *Alstonla boonei*. *International J. Phytopharmacology*. **1(2):** 119-123.

Atal, C. K., Sharma, M. L., Kaul, A. and Khajuria, A. 1986. Immunomodulating agent of plant origin. *Indian Drugs*. **18(2)**: 133-141.

Ballal, D., Srujan, K. K. Bhat, Shirwalkar, A. and Shivananda, P. G. 2001. Antibacterial activity of *Holarrhena antidysenterica* (Kurchi) against the enteric pathogens. *Indian J. Pharmacology*. **33:** 392-393.

Barriada-Pereira, M., Concha-Graña, E., González-Castro, M. J., Muniategui-Lorenzo, S., López-Mahía, P., Prada-Rodríguez, D. and Fernández-Fernández, E. 2003. Microwave-assisted extraction versus Soxhlet extraction in the analysis of 21 organochlorine pesticides in plants. *Elsevier B. V. J. Chromatogr.* **1008:** 115-122.

Bhutani, K. K. 1984. Proceedings of National symposium of allied biotechnology of medicinal, aromatic and timber yielding plants, University of Calcutta, India, p. 387.

Castello, M., Phatak, A., Chandra, N. and Sharon, M. 2002. Antimicrobial activity of crude extract from plant part and corresponding calli of *Bixa orellana* L. *Indian J. Exp. Biol*. **40:** 1378-1381.

Cetin, T. E. and Gurler, N. 1989. Bakterilerin antibiyotiklere duyaarlilik deneyinin yapilmasi. *Kukem Dergisi*. **12:** 2-5.

Chakraborty, A. and Brantner, A. H. 1999. Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubscens*. *J. Ethnopharmacology*. **68:** 339-344.

Chopra, R. N., Chopra, I. C., Handa, K. L. and Kapur, I. D. 1982. Chopras Indigenous drugs of India. Academic Press, New Delhi, India, p.342. *Indian J. Chem*. **20B:** 62.

Dadsena, R., Sahu, N. K., Agrawal, S. and Kumar, A. 2013.

Phytochemical analysis of three endangered plants (*Costus specious, Gloriossa superba* Linn and *Rauvolfia serpentine* (Linn) Benth from Kanker district of Chhattisgarh, India. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 655-659.

Dandapat, S., Kumar, M., Kumar, A. and Sinha, M. P. 2013. Antipathogenic efficacy of methanolic leaf extract of *Cinnamomum tamala* and *Aegle marmelos* with their nutritional potentiality. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 635-641.

Evans, J. S., Pattison, E. and Morris, P. 1986. Antimicrobial agents from plant cell culture, In: Secondary metabolites in plant cell culture. Morris P, Scraggs A, Stanford A, Fowler M. (Eds.). *Cambridge University, London*. p.12.

Ghosh, K. C. 1984. *Holarrhena antidysenterica* and *Wrightia tinctoria*. In: "Drugs of Hindustan"Hahnemann Publishing Co. Pvt. Limited, Calcutta. pp. 309-320.

Hagers Handbuch der Pharmazeutischen Praxis 1976. Springer-Verlag, Berlin. **V:** pp. 92-95.

Harborne, J. B. (eds). 1998. Phytochemical Method: A guide to modern techniques of plant analysis. 3rd. chapman and Hall, London ISBN: 0412-57270-2, p. 302.

Heble, M. R., Narayanswamy, S. and Chadha, M. S. 1976. Metabolism of cholesterol by callus culture of *Holarrhena antidysenterica*, *Phytochemistry*. **15:** 1911-1912.

Hossain, M. S., Ferdous, A. J. and Hasan, C. M. 1993. *In vitro* antimicrobial activity of alkaloids from the stem bark of *Desmos longiflorus* Roxb. *Bangladesh J. Bot*. **22:** 34-40.

Kirtikar, K. R. and Basu, B. D. 1994. Indian Medicinal plants, Dehradun, Shiva offset press; pp. 1707-1709.

Kullu, A. R., Tabassum, W. and Sinha, M. P. 2013. Effect of *Psidium guajava* aqueous extracts on haematological profile and serum lipid variables of albino rat. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 7437-46.

Kumar, A., Kumar, M., Dandapat, S. and Sinha, M.P. 2013. Antioxidant activity and pharmocological screening of *Tinospora cordifolia*. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 689-693.

Kumar, M., Kumar, A., Dandapat, s. and Sinha, M. P. 2013. Phytochemical screening and antioxidant potency of *Adhatoda vasica* and *Vitex negundo. The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 723-730.

Lin, J., Opoku, A. R., Geheeb-Keller, M., Hutchings, A. D., Terblanche, S. E., Jager, A. K., Van Staden, J. 1999. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and antimicrobial activities. *J. Ethnopharmacol*. **68:** 267-274.

Mungole, A. J., Zanwar, P. and Chaturvedi, A. 2008. Antibacterial and phytochemical screening from different parts and calli of *Physalis minima* (L.). *The Bioscan*. **3(4):** 393-395.

Murashige, T. and Skoog, F. 1962. Revised medium for rapid growth and Bioassays with tobacco tissue culture. *Physiol. Plant.* **15:** 473-497.

Panda, A. K., Bisaria, V. S., Mishra, S., Bhojwani, S. S. 1991. *Cell culture of Holarrhena* antidysenterica: growth and alkaloid production. **30(3):** 833-836.

Parekh, J. and Chanda, S. 2006. *In vitro* antimicrobial activities of extract of *Launaea procumbens* Roxb.(Labiateae), *Vitis vinifera* (Vitaceae) and *Cyperus. rotundus* (Cyperaceae). *Afr. J. Biomed. Res.*, **9:** 89-93.

Parekh, J., Nair, R. and Chanda, S. 2005. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J. Pharmacol*. **37:** 408-409

Patil, P. K. and Ghosh, J. S. 2010. Antimicrobial Activity of *Catharanthus roseus* – A Detailed Study. *British J. Pharmacology and Toxicology* **1(1):** 40-44.

Perez C, Paul M, Bazerque P. 1990. Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp*. **15:** 113-115.

Pretorious, C.J. and Watt, E. 2001. Purification and identification of active components of *Capobrotus edulis* L. *J. Ethnopharm*. **76:** 87-91.

Purohit, S. S. and Mathur, S. K. 1999. *Drugs in biotechnology fundamentals and applications*, Maximillan publisher, India. p. 576.

Radt, F. 1965. Elseviers encyclopaedia of organic chemistry, vol. 14.5. Springer-Verlag, New York. p. 4140.

Raha, S. and Roy, S. C. 2003. Efficient plant regeneration in *Holarrhena antidysenterica* Wall., from shoot segment-derived callus. *In Vitro Cellular and Developmental – Biology-Plan.* **39**(2)**:** 151-155.

Raman, M. S., Anwar, M. N., Begum, J. and Chowdhury, J. U. 1997. Antimicrobial activities of the alkaloids of three plant leaves. *Bangladesh J. Bot*. **26:** 79-81.

Raman, Md. S., Sultana, N. and Anwar, M. N. 2004. *In vitro* antimicrobial activity of Holarrifine-24ol isolated from the stem bark of *Holarrhena antidysenterica*. *International J. Agriculture and Biology***. 6(4):** 689-700.

Reddy, B. U. 2010. Antimicrobial Activity of *Thevetia Peruviana* (Pers.) K. Schum. and Nerium Indicum Linn.*The Internet J. Pharmacology*. **8(2):**

Sahu, P. R. and Sinha, M. P. 2013. Screening of antibacterial activity of crude leaf estracts of *Cassia tora* on UTI pathogens. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 735-738.

Sarkar, S. D., Muniruzzaman, S. and Khan, S. I. 1991. Antimicrobial activity of *Piper chaba* Hunter (Chui).*Bangladesh J. Bot*. **20:** 179-82.

Sharif, M. D. M. and Banik, G. R. 2006. Status and utilization of medicinal plants in Rangmati of Bangladesh. *Res. J. Agric. Biol. Sci.* **2(6):** 268-273.

Shariff, N., Sudarshana, S. M., Umesh, S. and Hariprasad, P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *African J. Biotechnology*. **5(10):** 946-950.

Sharma, P. C. Yelen, M. B. and Dennis, T. J. 2004. In database on medicinal plants used in Ayurveda, Vol **2**. New Delhi; *Central Council for Research in Ayurveda and Siddha,* p. 550.

Suffredini, I. B., Bacchi, E. M., Sakuda, T. M. K., Ohara, M. T., Younes, R. N. and Varella, A. D. 2002. Antibacterial activity of Apocynaceae extracts and MIC of *Tabernaemontana angulata* stem organic extract**,** *Brazilian J. Pharmaceutical Sciences.* **38(1):**

Tabassum, W., Kullu, A. R. and Sinha, M. P. 2013. Effects of leaf extracts of *Moringa oleifera* on regulation of hypothyroidism and lipid profile. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 665-669.

Thappa, R. K., Tikku, K., Saxena, B. P., Vaid, R. M. and Bhutani, K. K. 1989. Conessine as a larval growth inhibitor, sterilant, and antifeedant from *Holarrhena antidysenterica*. Insect Sci. Appl. **10(2):** 149-156.

Toppo, K. I., Gupta, S., Karkun, D., Agrawal, S. and Kumar, A. 2013. Antimicrobial activity of *Sphagneticola trilobata* (L.) Pruski, against some human pathogenic bacteria and fungi. *The Bioscan.* **8 (2): Supplement on Medicinal Plants.** 695-700.

Warrier, P. K., Nambier, V. P. and Ramankutty, C. 1994. Indian medicinal plant: a compendium of 500 species. *Delhi: orient longman*. pp.191-194.