ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 11, pp.9909-9914, November, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF *COLEUS AMBOINICUS* ON SIX BACTERIAL STRAINS

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ARTICLE INFO

Article History:
Received 19th August, 2014
Received in revised form
22nd September, 2014
Accepted 15th October, 2014
Published online 18th November, 2014

Key words:
Coleus amboinicus,
Antimicrobial activity,
Ethanolic extract,
Minimum inhibitory concentration.

ABSTRACT

Coleus amboinicus is a commonly available medicinal herb in India. Ethanolic extract of *Coleus amboinicus* leaves were used for study of antimicrobial activity. The antimicrobial activity was evaluated against six bacterial strains with four different concentrations by detecting minimum inhibitory concentration and zone of inhibition. The highest antibacterial activity was observed in *Salmonella typhi* at a concentration of 1000µg/ml. The lowest antibacterial activity was observed in *Staphylococcus aureus* at a concentration of 250µg/ml of ethanolic extract of *Coleus amboinicus* leaf extract.

INTRODUCTION

India is rich in indigenous herbal resources permitting the growth of more than 20,000 plant species of which, about 2,500 are of medicinal value (Choudhari and Maheswari, 2009). The medicinal value of these plants lies in some active chemical substances called phytochemicals that produce a definite physiological action of human body. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significances in therapeutic treatment (Arunkumar and Muthuselvam, 2009). Alkaloids, flavanoids, phenolic compounds and tannins are the major phytochemicals present in this plants (Edeoga *et al.,* 2005). *Coleus amboinicus*, Karpooravalli in Tamil is a country borage plant. It is also referred as *Plectranthus amboinicus*. It belongs to the family lamiaceae (Labiatae) and is also called as country borage in English (Kirtikar and Basu, 1999). It is a large succulent aromatic perennial herb found throughout India, Moluccas and Ceylon (Warrier and Nambier, 1996, Nadkarni, 2002). It is a medicinal plant, widely used in the Indian system of medicine. Many pharmacological properties have been reported including urolithriasis, antiepileptic, antitumorogenic, antimutagenic, radioprotective effect, antiviral, antifungal and neuropharmocological properties (Patel *et al.,* 2010). Afaf Mohammed Weli *et al.* (2011) found out twenty six constituents present in the essential oil of C*oleus aromaticus* growing in Oman. The major constituent being M-thymol (63.4%) followed by terpinene (11.8%), P-cymene (7.7%), Caryophyllene (7.1%) and beta – selinene (2.7%). The oil showed potent antibacterial activity against all tested micro organisms but it did not show any antifungal activity.

Synthetic antioxidants and antimicrobials in use have been shown to have harmful side effects (Gao *et al.,* 1999; Williams *et al.,* 1999 and Osawa and Namiki, 1981); therefore there is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources. Several medicinal plants with ethno – botanical uses have been used traditionally in the treatment of disease and have been exploited for these desired traits (Patel *et al.,* 2010, Okoro *et al.,* 2010; Lagnika *et al.,* 2011). Consequently there has been a growing interest to identify natural antioxidants and antimicrobials from these plants (Chanda and Dave 2009; Rice, 2004). Khory and Katrak (1999) reported that the decoction of *Coleus amboinicus* leaves is used in cases of chronic cough and asthma, and also as an antispasmodic, stomachic, and for the treatment of headache, fever, epilepsy and dyspepsia (Morton, 1992). It is also used in the treatment of skin ulcerations and urinary diseases, as well as to alleviate inflammation, kidney

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troubles, nervous disorders and in conditions of congestive heart failure (Franca *et al.,* 1996; Jain and Lat, 1996). Lukhoba *et al.* (2006) recently reviewed the ethanobotanical uses of *P.ambonicus.*

Malini *et al.* (2013) studied antimicrobial activity of Ethanolic and Aqueous extracts of *Aloe vera* and *Coleus aromaticus* against waste water pathogens like *E. coli, Pseudomonas sp*, *Klebsiella sp, Bacillus sp* and *Styphylococcus sp.* They found out minimum antibacterial activity of 6.66 ± 0.667 mm zone of inhibitions in aqueous extract and maximum antibacterial activity of 20.50± 0.500 mm zone of inhibition in ethanolic extract. They further confirmed the ethanolic extracts of *Aloe vera sp* showed the maximum activity 22.46± 0.2990 mm zone of inhibition against waste water pathogen. Nivya Mariam Paul *et al.* (2014) studied phytochemical screening and antimicrobial activity of *Coleus amboinicus* against twelve different bacterial strains. Phytochemical analysis of ethanolic extract of fresh leaves showed the presence of flavanoids, terpenoids, phenolic compounds tannins and cardiac glycoside. Ethanolic extracts of shade dried and oven dried leaves showed the presence of terpenoids, phenolic compounds, tannins and cardic glycoside. Aqueous extract of fresh leaves showed the presence of Alkaloids, Flavanoids, phenolic compounds and tannins and gum. Aqueous extract of shade dried leaves showed the presence of alkaloids, phenolic compounds, tannins and gum. Aqueous extract of oven dried leaves showed the presence of phenolic compounds, tannins, morphine alkaloids and gum. This work was supported by the work of Roshen Patel *et al.* (2010) they detected the presence of alkaloids, carbohydrates, glycosides, proteins aminoacides, terpenoids, quinine, tannins and flavanoids in *Coleus amboincus* leaves.

They further found out the ethanolic leaf extract of *coleus amboinicus* showed more antibacterial activity against all the bacterial strains than the aqueous leaf extract of *Coleus amboinicus*. Melva Silitonga *et al.* (2014) found out Bangun bangun (*Plectranthus amboinicus* Lour) increased the productivity of breast milk in black tribe woman who consumed *Plectranthus amboinicus* just gave birth in North Sumatra Indonesia. This plant is known to have a high content of nutrients, especially iron carotene. Also known to have many benefits among other as an antipyretic, analgesic, wound medicine, cough medicine and thrush, antioxidant, antitumour, anticancer and hypotensive

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Coleus amboinicus* collected from Kanchipuram District, Tamil Nadu. It was authenticated by Dr. P. Jayaraman Director, Plant Anatomy Research Center Thambaram Chennai. Retired Professor of Botany. Presidency College, Chennai, TamilNadu. (Reg.No. PARC/2013/2081) The plants were freshly collected and the leaves were separated from the stem, washed with running tap and rinsed in distilled water. The leaves were shade dried after complete dryness they were ground well to fine powder and then transferred into airtight containers.

Extraction

The leaves were chopped into small pieces and were shade dried for 2 weeks. The dried leaves were powdered, using mechanical grinder. 20g of the plant powder sample was ground and soaked with ethanol in a 250 ml conical flask. The flask was plugged with cotton wool and aluminium foil to prevent the solvent from escaping. The flask was placed in a shaker for 24 hrs. The filtrate was concentrated in a rotory evaporator to get the crude plant extracts.

Antimicrobial screening

Bacteria: *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherchia coli, Klebsiella pneumoniae* and *Proteus species*. The bacterial cultures were maintained in Nutrient Agar (NA) slants at 4^{o}C.

Bacterial inoculums preparation

Bacterial cultures used in this study were obtained from Microbial Type Culture Collection (MTCC).Lab Chandigarh, India. Bacterial cultures included in this study were *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa, Escherchia coli*, *Klebsiella pnuemoniae* and *Proteus species*. All the cultures were grown in Muller – Hinton broth medium. The inoculums was used for antibacterial assay. Stock cultures were maintained at 4^{o}C on nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 37^{o}C for 24 hrs.

Antibacterial assay

The media and the test bacterial cultures were poured into dishes (Muller – Hinton agar media). The test strain (0.2 ml) was inoculated into the media to inoculums size (108 cells / ml) when the temperature reached 40-42^{o}C care was taken to ensure proper homogenization. The plant extracts was tested for antibacterial activity in the agar well diffusion assay against *Staphylococcus aureus*, *Escherchia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia* and *Proteus spp*.

Agar well Diffusion Method

The inoculums of microorganism was prepared from pure culture according to the method of Parihar and Bohra, (2006). The antibacterial activity of plant extract was determined by Disc Diffusion Method of Muller Hinton Agar (MHA) medium (Bauer *et al.,* 1966). The Muller Hinton Agar medium is poured into the pertiplate, after solidification the inoculums, were spread on the solid plates with sterile swab moistened with the bacterial suspension. The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) and extracts were loaded on the 6 mm diameter, sterile disc (Himedia, Bombay) with the different concentration.

The disc were placed in MHA plate. Different concentration of leaf extract (1000µg/ml, 750µg/ml, 500µg/ml and 250µg/ml)

were added to the bacterial suspension. The samples were placed in the disc. The plates were incubated for 24 hrs at 37°C. DMSO (20µg/disc) was used as control. Ampicillin (100 µg) was used as standard. The inhibition zone around the disc (diameter) was measured and recorded. The antimicrobial activity was determined by measuring the diameter of zone of inhibition.

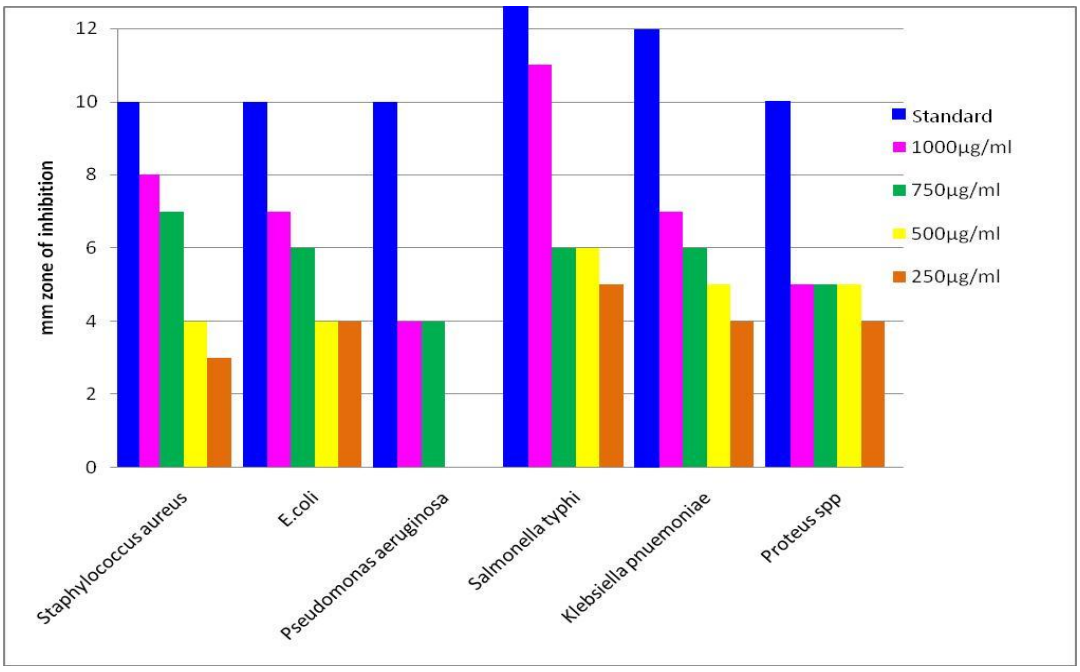
RESULTS

Antibacterial activity of *Coleus amboinicus* was analyzed against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella pneumoniae* and *Proteus species*. The *Staphylococcus aureus* showed 8 mm zone of inhibition in 1000µg/ml, 7 mm zone of inhibition in 750µg/ml, 4 mm zone of inhibition in 500µg/ml and 3 mm zone of inhibition in 250µg/ml of leaf extract of *Coleus amboinicus*. The E.coli showed 7 mm zone of inhibition in 1000 µg/ml, 6 mm zone of inhibition in 750µg/ml, 4 mm zone of inhibition in 500µg/ml and 4 mm zone of inhibition in 250µg/ml of leaf extract. *Pseudomonas aeruginosa* showed 4 mm zone of inhibition in 1000µg/ml, 4 mm zone of inhibition in 750µg/ml. No inhibition were observed in the concentration of 500µg/ml and 250µg/ml of leaf extract against *Pseudomonas aeruginosa.*

On the other hand *Salmonella typhi* showed 11 mm zone of inhibition in 1000µg/ml, 6 mm zone of inhibition in 750µg/ml, 6 mm zone of inhibition in 500µg/ml and 5 mm zone of inhibition in 250µg/ml of leaf extract. *Klebsiella pnuemoniae* showed 7 mm zone of inhibition in 1000µg/ml, 6 mm zone of inhibition 750µg/ml, 5 mm zone of inhibition in 500µg/ml and 4 mm zone of inhibition in 250µg/ml of plant extract. *Proteus species* showed 5 mm zone of inhibition in 1000µg/ml, 750µg/ml and 500µg/ml and 4 mm zone of inhibition was found in 250µg/ml of *Coleus amboinius* leaf extract. The highest antimicrobial activity was observed in *Salmonella typhi* (11 mm zone of inhibition) at 1000µg/ml of leaf extract, whereas lowest antimicrobial activity was observed in *Staphylococcus aureus* (3 mm zone of inhibition) at 250µg/ml of concentration of leaf extract. But in *Pseudomonas aeruginosa* showed no antibacterial activity at 500µg/ml and 250µg/ml of leaf extract. In standard treated with Amphicillin (100 µg) showed 10mm zone of inhibition in *Staphylococcus aureus*, *E.coli, Pseudomonas aeruginosa* and *Proteus spp.*20mm zone of inhibition in *Salmonella typhi* and 13mm zone of inhibition in *Klebsiella pnuemoniae.* The results are presented in Table -1, Bar graph-1 and Figure 1 – 6.

Table 1. Screening of antibacterial activity of *Coleus amboinicus* leaves extracts against different bacterial strains

S.No	Micro Organisms	Concentration (µg/ml)				Std	DMSO
		1000	750	500	250		
1.	*Staphylococcus aureus*	8	7	4	3	10	-
2.	*E.coli*	7	6	4	4	10	-
3.	*Pseudomonas aeruginosa*	4	4	-	-	10	-
4.	*Salmonella typhi*	11	6	6	5	20	-
5.	*Klebsiella pnuemoniae*	7	6	5	4	13	-
6.	*Proteus spp*	5	5	5	4	10	-



Bar graph-1. Screening of antibacterial activity of *Coleus amboinicus* leaves extracts against different bacterial strains

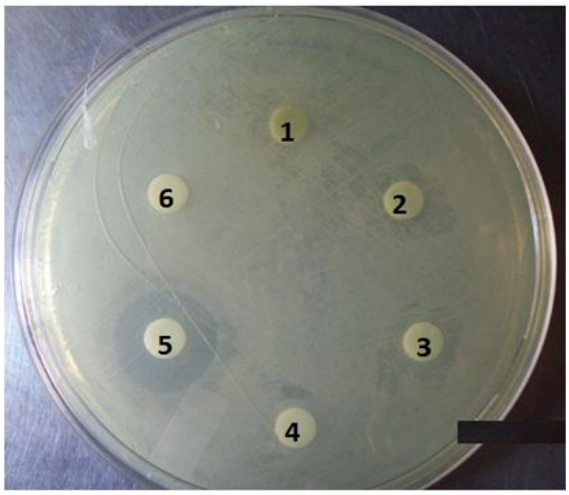


Fig.1. Antibacterial activity of *Coleus amboinicus* leaves extracts against *Staphylococcus aureus*

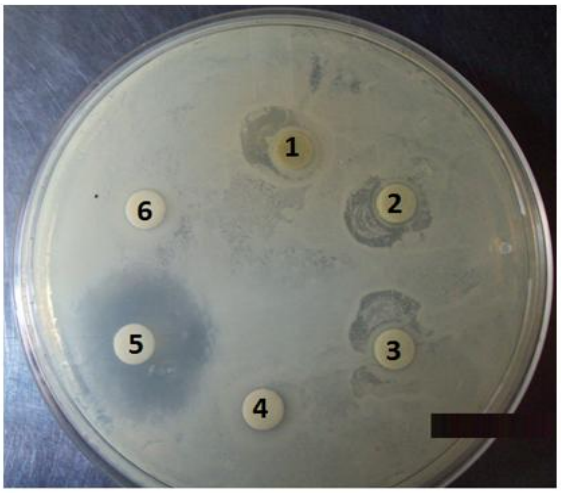


Fig. 2. Antibacterial activity of *Coleus amboinicus* leaves extracts against *E.coli*

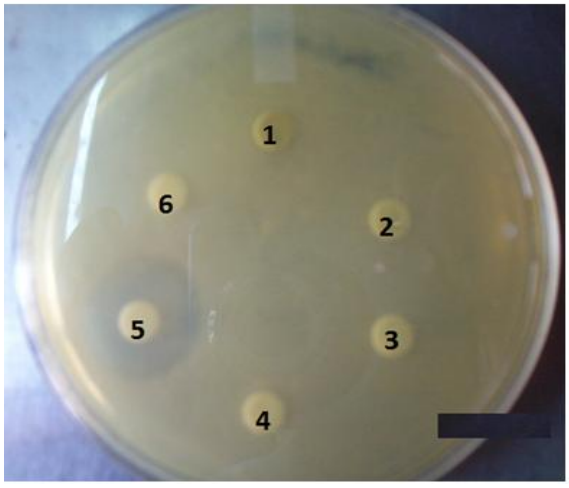


Fig.3. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Pseudomonas aeruginosa*

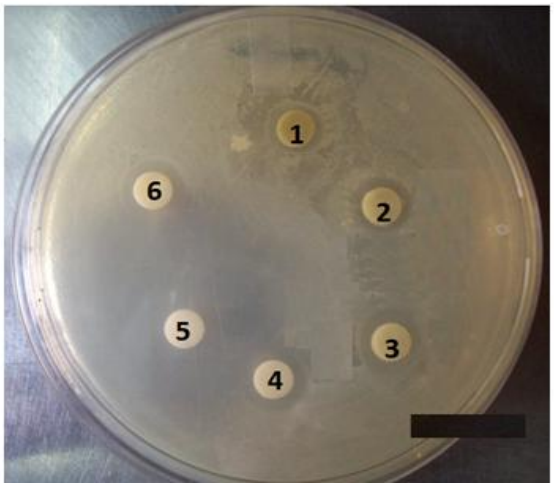


Fig.4. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Salmonella typhi*

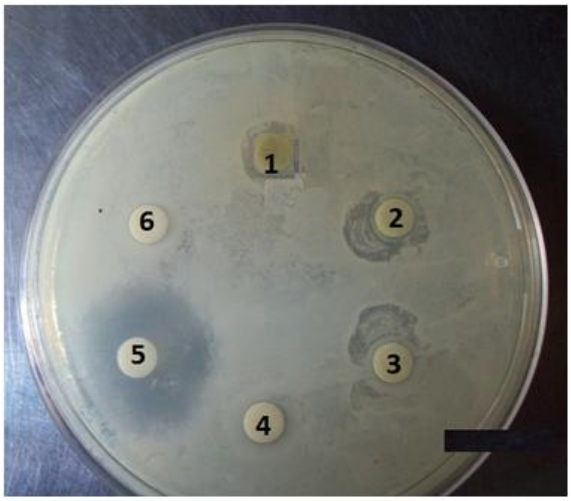


Fig.5. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Klebsiella pnuemoniae*

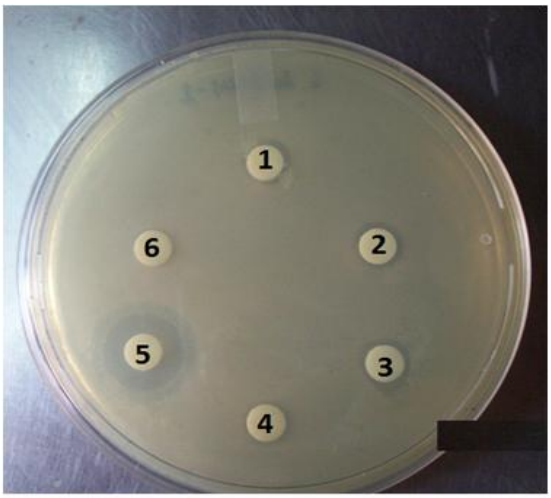


Fig.6. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Proteus spp*

Nuumers in the agar plate indicates the concentration of the leaf extract.

1. *Coleus amboinicus* : 1000 µg
2. *Coleus amboinicus* : 750 µg
3. *Coleus amboinicus* : 500 µg
4. *Coleus amboinicus* : 250 µg
5. Standard – Amphicillin : 100 µg
6. DiMethyl Sulphoxide (DEMSO):20 µg

DISCUSSION

Plants are important source of potentially useful structure for the development of new chemotherapeutic agents (Tona *et al.,* 1998). The ethanolic crude extract of *Coleus ambonicus* leaf showed the highest antimicrobial activity against *Salmonella typhi* 11 mm and *Staphylococcus aureus* 8 mm zone of inhibitions at 1000 µg/ml concentration. The results are in agreement with those of Shiney ramya *et al.* (2012). They concluded that alcoholic crude extract of *Coleus aromatics* and *Leucas aspera* leaves were exhibited antibacterial activity against *Shigella sp*, *Salmonella typhi* and *E. coli*, this is due to the presence of antimicrobial substances in the leaf extract like tannis, alkaloids and glycosides. Roa *et al.* (1991) reported that oil isolated from the leaves of *Coleus amboinicus* exhibited varying degree of antimicrobial activity against a number of pathogenic and non-pathogenic Fungai and Bacteria. Prudent *et al.* (1995) also analyzed the essensial oil of *Coleus aromaticus* for its bacteriostatic and fungistatic properties. The results are further supported by the work of Subhas Chandrappa *et al.* (2010). They found out ethanol extract of *Coleus aromaticus* showed strongest antibacterial activity with the MIC value of 25-39µg/ml against 3 Gram positive and 2 Gram negative human pathogenic bacteria. Ethanol extract of *Coleus aromatius* (ECA) showed more potential of antibacterial effect than Hot water *Coleus aromaticus* extract (HWCA). This may be because the alcohol extract is rich in polyphenol and other bioactive compoundes, which are responsible for its antibacterial activities.

The work of Pritima and Pandian (2008) is of great interest. They found the *Coleus ambonicus* species growing in India have more effective in antibacterial and antifungal activities. They conducted an experiment, the *Coleus ambonicus* plant was tested on micro-organism causing reproductive tract infections (RTI) in females. When tested against *Candida Krusei* there was the highest 20 mm zone of inhibition, followed by *C. albicans, Proteus mirabilis, E.coli, S.aureus, E.faecalis, Klebsiella pneumoniae* and the least inhabitation was observed on *Neisseria gonarreae*. In the present study the Lamiaceae (Labiatae) leaf extract from Kanchipuram District, Tamilnadu had greater on the diameter of the inhibition zone for *S. aureus* than *E. Coli*, indicating that the gram positive strain is more sensitive than the gram negative strain. The leaf extract from Tamilnadu having inhibitory effect on the gram positive bacteria (*Staphylococcus aureus*) compared to the gram negative bacteria (*Escherichia coli* and *Pseudomonas aruginosa*. Generally gram negative bacteria are more resistance to plants extract compared to gram positive bacteria (Basri and Fan, 2005). This may be due to the permeability barrier provided by the cell wall. The reason is that the density of the lipopolysaccharide layer in the outer surface of bacterial cell wall, which is greater in the gram negative bacteria as compared to the gram positive bacteria (Burn, 1988). It has been concluded that the antibacterial properties is of due to nucleotide damage with increase in spatial division and condensation of genetic material (Chakruborty *et al.,* 2007).

The results proves that the use of the leaves of *Coleus amboinicus* to cure several illness, especially those caused by microbes, is valid.

REFERENCES

Afaf Mohammed Weli, Dina Selim, Mohammad Sohail Akhtar, Jamal Nasser Al - Sabahi, Qasim Al –Riyami, Zakia Mubarak Al-Sulimi, Samera Suliman Adi Al-Salmani, Basema Obaid, Suliman Al –Khanjari 2011. Phytochemical Investigations and Antimicrobial screening of *Coleus aromaticus* grown in Oman. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* 10(11) 3083- 3090.

Arunkumar. S and Muthuselvam 2009. Analysis of phytochemial constituents and Antimicrobial Activities of *Aloevera* L. against clinical pathogens. *World Journal of Agricultural Science,* 5 (5): 572-576.

Basri. D.F and Fan S.H 2005. The potential of aqueous and acetone extracts of *galls of Querus infectoria* as antibacterial agents. *Indian J. Pharmacol*., 37 (1), 26-29.

Bauer A.W, Kirby W.M, Sherris J.C and Jurc K.M. 1996. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.,* 45 (4) : 493-496.

Burn, P. 1988. Talking point Amphitropic proteins; a new class of membrane proteins. *Trends in Biochemical Science, 1*3 (3), 79-83.

Chakraborty, D, Mandal S.M., Chakraborty J., Battacharuaa. P.K., Bandyopandhyay, A., Mitra A and Guptu, K. 2007. Antimicrobial activity of leaf extract of *Basillicum polystauchyan* (L) Monech. *Indian. J. Exp. Biol*., 45, 744-748.

Chanda, S. and R. Dave, 2009. *"In Vitro* Models for Antioxidant Activity Evaluation and Some Medicinal Plants Possessing Antoxidant Properties – An Overview," *African Journal of Microbiology Research,* Vol. 3, No. 13, pp.981-996.

Choudhari, M.M. and Maheswari, J.K. 2009. Ethnobotany in South Asia. *Middle East Journal of Science Research,* 4 (3): 144-146.

Edeoga HO, Okwu D.E and Mbacbie 2005. Phyto chemical constituents of some Nigerian Medicinal plants *African Journal Biotechnology,* 4 (7): 685-688.

Franca, F, E.L. Lago and P.D. Marsden, 1996. "Plants Used in the Treatment of Leishmanial Ulcers Due to *Leishmania* (Viannia) *braziliensis* in an Endemic Area of Bahia, Brazil", *Revista-Sociedade Brasileira de Medicinal Tropical,* Vol. 29, No.3, pp.229-232.

Gao, J.J, K. Igalashi and M. Nukina, 1999. "Radical Scavenging Activity of Phenylpropanoid Glycosides in *Caryopteris Incana," Bioscience, Biotechnology and Biochemistry,* Vol.63, No.6, pp. 983-988.

Jain, S.K and S. Lata, 1996. "Amazonian Uses of Some Plants Growing in India", *Indigenous Knowledge and Development Monitor,* Vol. 4, No.3, pp.21-23.

Khory, N.R and N.N. Katrak, 1999. "Materia Medica of India and Their Therapeutics," BDH Printers, New Delhi, p.380.

Kirtikar. K.R and B.D. Basu, 1999 "Indian Medicinal Plants," Vol.3, *International Book Distributors, Dehradun*, pp. 1970-1971.

Lagnika, L, E. Anago and A. Sanni, 2011. "Screening of Antibacterial, Antioxidant Activity and Toxicity of Some Medicinal Plants Used in Benin Folkloric Medicine," *Journal of Medicinal Plants Research*, Vol. 5,No. 5, pp. 773-777.

Lukhoba, C.W, M.S.J. Simmonds and A.J. Paton, 2006. "*Plectranthus*: A Review of Ethanobotanical Uses," *Journal of Ethanopharmcology,* Vol. 103, No.1, pp.1-24.

Malini, M, G. Abiraimi, V. Hemalatha and G. Annadurai, 2013. Antimicrobial activity of Ethanolic and Aqueous Extracts of medicinal plants against waste water pathogens. *International Journal of Research in Pure and Applied Microbiology,* 3(2):40-42

Melva Silitonga, Syafruddin Ilyas, Salomo Hutahaean and Herbert Spinahuter 2014. Levels of Apigenin and Immunostimulatory activity of leaf extract of Bangun bangun (*Plectranthus amboinicus* lour). *International Journal of Biology,* vol. 7(1); 46-53

Morton, J.F 1992. "Country Borage (*Coleus aromaticus* Lour): A Potent Flavouring and Medicinal Plant," *Journal of Herbs, Spices and Medicinal Plants,* Vol. 1, No.1-2, p.77.

Nadkarni, 2002. "Indian Materia Medica," 3rd Edition, Popular Prakashan, Mumbai, pp.371-372

Nivya Mariam Paul, Anjali, Mohan, Elza John, Nayomi John, Ajitha. A.R and Shanthi A Avirah. 2014. Phytochemical, screening and evaluation of the anti microbial potential of *Coleus amboinicus*. *World Journal of Pharmacutical Research*, Vol 3(2); 2811-2826.

Okoro, I.O, A. Osagle and E. O. Asibor, 2010. "Antioxidant and Antimicrobial Activities of Polyphenols from Ethanomedicinal Plants from Nigeria," *African Journal of Biotechnology,* Vol. 20, pp.2989-2993.

Osawa, T. and M. Namiki, 1981. "A Noval Type of antioxidant isolated from Leaf Wax of *Eucalyptus* Leaves," *Agriculture Biology and Chemistry,* Vo. 45, No. 3, pp.735-739.

Parihar, L and A. Bohra, 2006. Antimicrobial activity of Stem extracts of some species plants. *Ad Plant Science,* 19(II).

Patel, R, N.K. Mahobia, R. Ghendle, B. Kaushik and S. K. Singh, 2010. "Diuretic Activity of Leaves of *Plectranthus amboinicus* (Lour) Spreng in Male Albino Rats," *Pharmocology Research,* Vol. 2, No. 2, pp.86-88.

Patel, V.R, P. R. Patel and S. S. Kajal, 2010. "Antioxidant Activity of Some Selected Medicinal Plants in Westerns Region of India," *Advances in Biological Research,* Vol.4, No.1, pp.23-26.

Pritima .R.A and R.S. Pandian, 2008. Antimicrobial activity of *coleus aromaticus* (benth) against microbes of reproductive tract infections among women. *African Journal of Infectious Diseases,* 1 (1) 18-24.

Prudent, D, Perineau, F, Bersiere J.M, Michel G.M and Baccou J.C 1995. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus benth*) evaluation of its bacteriostatic and Fungistatic properties. *J. Esst. Oil Research,* 7; 165-173.

Rao, A, GSJG, Baby, P and Prasad R.Y. 1991. Leaf oil of *Coleus amboinicus* Lour: the *in vitro* antimicrobial studies. *Perfume and Kosmetik,* 725 (11); 744-745.

Rice-Evens, C.A. 2004. "Flavonoids and Isoflavones: Absorption, Metabolism and Bioactivity," *Free Radical Biology and Medicine,* Vol. 36, No.7, pp.827-828.

Roshan Patel, Naveen M., Manjul PS, Gulzar A., Anita S and Sudarshan S. 2010. *Plectranthus Amboinicus* (Lour) Spreng : an Overview. *The Pharma Research,* (T.Ph.Res.) 4; 01-15.

Shiney ramya, B, P. Ganesh and R. Sureshkumar 2012. Phytochomical screening of *Coleus aurometicus* and *leucas aspera* and their Antibacterial activity against Enteric Pathogen. *International Journal of Pharmaceutical of Biological Archives,* 3 (1) : 162-166.

Subhas Chandrappa, M., Harsha, R., Dinesha, R. and Thammanna Gowda, S.S. 2010. Antibacterial activity of *coleus arometicus* leaves. *International Journal of Pharmacy and Pharmaceutical Science,* Vol.2, (3) 63-66.

Tona, L, K. Kambu, N. Ngimbi, K. Cimanga and A.J. Vlietinck 1998. Antiamoebic and Phytochemial screening of some Congolese medicinal plants *J. Ethnopharmacol*., 61; 57-65.

Warrier, P.K. and V.P. Nambier, 1996. "Indian Medicinal Plants: A Compendium of 500 Species," Vol.4, Orient Longman Ltd., Chennai, p.315.

WHO 2002. Traditional Medicine Growing Needs and Potential. WHO policy perspectives on Medrainesa Geneva pp.1-6.

Williams, G.M, M.J. Iatropoulos and J. Whysner, 1999. "Safety Assessment of Butylated Hydroxyanisole and Butylated Hydroxyltoluene as Antioxidant Food Additives," *Food Chemistry and Toxicology,* Vol. 37, P.p.1027-1038.

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