

Copyright © 2015 Desai and Desai
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORIGINAL RESEARCH

HPTLC and bioautography analysis of *Casuarina equisetifolia* L. and *Sphaeranthus indicus* extracts against *Salmonella* spp.

Jalpa J. DESAI^{1*}, Piyush V. DESAI¹

¹Department of Biosciences, Veer Narmad South Gujarat University,
Surat – 395007, Gujarat, India

*Corresponding author email: j.d.200211@gmail.com

• Received: 15 June 2015 • Revised: 28 June 2015 • Accepted: 2 July 2015 • Published: 15 July 2015 •

ABSTRACT

Plants have been used for medicinal purpose since ancient times. Secondary metabolites of medicinal plants have shown to possess curing activity against different ailments as well as infections caused by microorganisms. Salmonellosis, a foodborne infection, is emerging as a life threatening disease because of drug resistant strains of *Salmonella* spp. In present investigations, plants *Casuarina equisetifolia* and *Sphaeranthus indicus* were found to possess significant inhibitory activity against *Salmonella* species. HPTLC and bioautography assay of methanolic extracts of *C. equisetifolia* indicated polyphenols as major active compounds against *S. typhi*. The present study indicates that the polyphenols of this plant can be used as a potential drug against infectious disease caused by *S. typhi*.

KEY WORDS: *Casuarina equisetifolia*, *Sphaeranthus indicus*, *Salmonella* spp., HPTLC, bioautography

Introduction

In developing countries, the extensive use of medicinal plants, because of their pharmacological properties, as primary remedies is very common (Conco, 1991). Plants such as *C. equisetifolia* L. (commonly known as 'saru' or 'ironwood') and *Sphaeranthus indicus* (locally known as 'gorakhmundi') are known to possess antimicrobial activity due to presence of bioactive phytoconstituents (Ogunwande *et al.*, 2011; Ramachandran, 2013).

During last two decades, foodborne diseases have emerged as a growing public health and economic problem in many countries. World Health Organization (WHO, 2005) reported that 1.8 million people died from diarrheal diseases largely due to contaminated food and water and *Salmonella* was reported to cause 31% of food related deaths (Sudershan *et al.*, 2014). *Salmonella* spp. causes Salmonellosis which is a life threatening disease. According to the CDC (Center for Disease Control) almost 21.5 million people in developing

countries contract typhoid each year. An estimated 600,000 deaths from enteric fever occur annually throughout the world (Gautam *et al.*, 2002; Ajayi *et al.*, 2010). Regardless of the existence of potent antibiotics against Salmonellosis, the appearance of resistant or multi-resistant strains, in addition to side effects of these antibiotics, imposes the need for a permanent search and development of new drugs (Friedman, 2015).

The present investigation was thus conducted to identify new plant based compounds against this fatal disease using *C. equisetifolia* and *S. indicus* extracts. In this regard, Primary screening was carried out to find out the activity and minimum inhibitory concentration of the active extract. Further, based on the primary phytochemical screening, high performance thin layer chromatography (HPTLC) was performed to separate the phytoconstituents from the crude extracts showing good activity against identified *Salmonella*

spp. Bioautography agar overlay bioassay on the developed HPTLC plates was carried out against *S. typhi* to screen and localize the separated group, based on their retention factor (Rf) value, for identifying antimicrobial compound. HPTLC analysis and bioautography assay give the clear idea about the phytochemical groups responsible for showing Anti-Salmonellae activity.

Materials and Methods

Collection of Samples

Fresh plant of *C. equisetifolia* was collected from Shree BapalalVaidhya Botanical Research Center, Veer Narmad South Gujarat University, Surat, and *S. indicus* was collected from the bank of river Tapi, Jahangirpura, Surat, Gujarat, India. Taxonomic identification of the plants was confirmed by the plant taxonomists of Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India. Plant parts were separated, washed under the running tap water and dried at 45°C in the oven. These dried parts were then homogenized to fine powder and stored in the air tight container to screen their activities against *Salmonella* species.

Preparation of Extracts

Methanolic extracts were prepared by, adding 10gm of plant powder to 250 ml of the solvents using Soxhlet extraction apparatus (Superfit Continental Pvt. Ltd.) for 12 hours at 65°C. Further, extracts were concentrated under vacuum in rotary evaporator to 1/10th of the initial volume. This solution is then used for the further investigation.

High Performance Thin Layer Chromatography

High Performance Thin Layer Chromatography of the methanolic extracts of both the plants was carried out on the CAMAG HPTLC System. Prior to sample application, HPTLC plate (HPTLC Silica gel 60 F254, Merck) was cut in the dimension of 8 x 10 cm. 10 µl of the crude extract was then applied as a single band of 8 mm length on the HPTLC plate using a CAMAG automatic TLC sampler III (CAMAG, Switzerland). Such three bands of a same sample were prepared on the plate for observing the activity against all three studied *Salmoenlla* spp. The plates were then subjected to 20 ml of standardized solvent system containing

Toluene: Ethyl acetate: Formic Acid (5: 4: 1) as per method described by Thube and Patil (2013) . After the successful separation, the plates were examined under the UV Chamber at 254 nm and 366 nm. Plates were then used for bioautography assay.

Microorganisms

The bacterial strains *Salmonella typhi* (MTCC 3216), *Salmonella paratyphi* A (MTCC 3219), *Salmonella enteritidis* (MTCC 3220) were used in the present study. They were procured from Microbial Type Culture Collection, Chandigarh, India.

Bioautography assay

Chromatograms were placed in 9 cm x9 cm sterile petri dishes with covers and exposed to UV light for 30 min. Overlay 10 ml Muller Hinton Agar (Medium No. 2, HiMedia, India) was distributed over the developed HPTLC plates. After solidification of the media, culture, at a final concentration of 10⁶ cells/ml, was spread on the surface of the media using sterile swabs. Plates were kept at 4°C for certain period of time for diffusion. These plates then incubated at 37°C for 24 hours. The bioautograms were flooded with 2% nitrobluetetrazolium chloride for observation of the inhibition zones (Saxena *et al.*, 1995).

Results and Discussion

Plants have been an enriched source of biologically active molecules and thus used as alternative medicines for the maintenance of human health against various ailments and infections (Farombi, 2003).

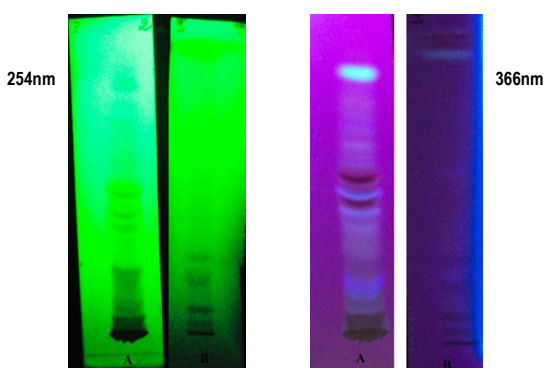


Figure 1: HPTLC chemoprofiling of the methanol extracts of (A) *S. indicus* and (B) *C. equisetifolia* under (a) 254 nm and (b) 366 nm

Present study was carried out to identify the potential phytoconstituent of *C. equisetifolia* and *S. indicus* having anti-*Salmonella* activity. For this study, methanol extracts of both the plants were used for HPTLC analysis and good separation of phytoconstituents was observed at 254 nm and 366nm (Fig. 1).

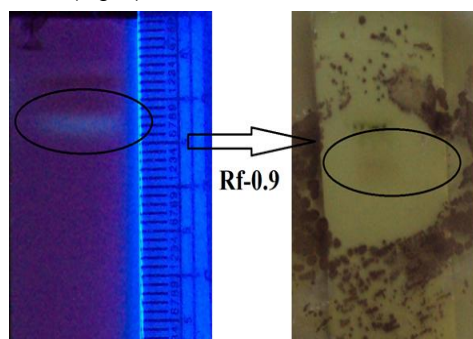


Figure 2: Bioautography on HPTLC chromatogram of *C. equisetifolia* showing zone of inhibition at Rf-0.9 against *S. typhi*.

Similar results were reported by Thube and Patil (2013). When overlaid with *S. typhi*, *S. paratyphi* A and *S. enteritidis*, zone of inhibition was observed only with *S. typhi* on the HPTLC chromatogram at Rf value 0.92 having blue intensified fluorescent band (Fig. 2). Similar Rf values were obtained for polyphenols in the investigation of antioxidant activity of three selected *Micromeria* species (Vladimir-Knezevic *et al.*, 2011). Also similar kind of bioautography assay results have been explained by Choma *et al.*, 2010.

Conclusion

Bioautography assay on HPTLC shows that methanolic extract of *C. equisetifolia* has higher Anti-Salmonellae activity as compared to *S. indicus*. Zone of inhibition around the polyphenol band depicts its bioactivity against *S. typhi* and proves its candidature for alternative medicament against Salmonellosis. Present finding clearly demonstrate that methanolic extract of *C. equisetifolia* can be used as a source of potent drug to cure various diseases caused by *Salmonella* species.

References

Ajayi, A. O., and Akintola, T. A. (2010). Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens. *African Journal of Microbiology Research*, 4(4), 314-316.

Choma, I. M., and Grzelak, E. M. (2011). Bioautography detection in thin-layer chromatography. *J Chromatogr A*, 1218(19), 2684-2691.

Conco, W. Z. (1991). Zulu traditional medicine: its role in modern society. *Community Health*, 5, 8-13.

Farombi, E. O. (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African Journal of Biotechnology*, 2(12), 662-671.

Friedman, M. (2015). Antibiotic-Resistant Bacteria: Prevalence in Food and Inactivation by Food-Compatible Compounds and Plant Extracts. *Journal of Agricultural and Food Chemistry*, 63(15), 3805-3822.

Gautam, V., Gupta, N. K., Chaudhary, U., and Arora, D. R. (2002). Sensitivity pattern of Salmonella serotypes in Northern India. *The Brazilian Journal of Infectious Diseases*, 6(6), 281-287.

Ogunwande, I. A., Flamini, G., Adefuye, A. E., Lawal, N. O., Moradeyo, S., and Avoseh, N. O. (2011). Chemical compositions of *Casuarina equisetifolia* L., *Eucalyptus torelliana* L. and *Ficus elastica* Roxb. ex Hornem cultivated in Nigeria. *South African Journal of Botany*, 77(3), 645-649.

Ramachandran, S. (2013). Review on *Sphaeranthus indicus* Linn. (Kot̄taikkarantai). *Pharmacognosy Reviews*, 7(14), 157-169.

Saxena, G., Farmer, S., Towers, G. H. N., and Hancock, R. E. W. (1995). Use of specific dyes in the detection of antimicrobial compounds from crude plant extracts using a thin layer chromatography agar overlay technique. *Phytochemical Analysis*, 6(3), 125-129.

Sudershan, R. V., Naveen Kumar, R., Kashinath, L., Bhaskar, V., and Polasa, K. (2014). Foodborne Infections and Intoxications in Hyderabad India. *Epidemiology Research International*, 5.

Thube, S. A., and Patil, M. J. (2013). Antiacne Effect of Polyherbal Gel Formulation in Male Sprague-Dawley Rats. *International journal of Research in Ayurveda & Pharmacy*, 4(3), 398-401.

Vladimir-Knezevic, S., Blazekovic, B., Stefan, M. B., Alegro, A., Koszegi, T., and Petrik, J. (2011). Antioxidant activities and polyphenolic contents of three selected *Micromeria* species from Croatia. *Molecules*, 16(2), 1454-1470.