International Journal of Current Research in
Biosciences and Plant Biology
Volume 5 • Number 3 (March-2018) • ISSN: 2349-8080 (Online)
Journal homepage: www.ijcrbp.com

Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2018.503.005

Antibacterial Activity of Ethanol Extracts of *Sesamum alatum* Thonn. Leaves

M. Sundarakumar[1] and N. Karmegam[2]*

[1, 2] Department of Botany, Government Arts College, Salem-636 007, Tamil Nadu, India
[1]II Year M.Sc., Botany Student.

***Corresponding author.**

Article Info

Date of Acceptance:
07 February 2018

Date of Publication:
06 March 2018

Keywords

Antibacterial activity
Inhibition zone
Leaf extract
Resazurin dye reduction
Sesamum alatum

ABSTRACT

In the present study the ethanol extract of *Sesamum alatum* Thonn. leaves were tested for antibacterial activity using disc diffusion assay followed by resazurin dye reduction test. The antibacterial activity of plant extracts was determined by resazurin dye reduction method. The dye resazurin was obtained from Sigma chemicals and prepared as 10g /l sterile water stock solution, stored in frozen at -20°C, thawed and diluted 1:10 in sterile water when required. The titreplate resazurin assay was performed in 96-well plates. Two-fold dilutions of each antibiotics and plant extracts were prepared in the test wells in complete nutrient broth, the final antibiotic concentrations being streptomycin 0.06 mg/l and tetracycline 0.12 mg/l. Twenty microlitres of each bacterial suspension was added to 180 µl of antibiotics and plant extracts containing culture medium. The minimum inhibitory concentration (MIC) of the ethanolic extracts of the leaves were rapidly assessed against five different bacterial species, *viz.*, *Micrococcus luteus* (NCIM-2103), *Staphylococcus epidermidis* (NCIM-2493), *Escherichia coli* (NCIM-2065), *Pseudomonas cepacia* (NCIM-2106) and *Bacillus megaterium* (NCIM-2187). The highest antibacterial activity with least MIC values were recorded in the ethanolic leaf extracts of *Sesamum alatum* against the test bacteria (MIC = 62.5-250 µL). The diameter of zone of inhibition was ranged from 0.9-1.9 cm for the bacteria tested. The highest inhibition zone was observed against *Escherichia coli* (1.9 cm) followed by 1.6 cm for *Pseudomonas cepacia* and 1.4 cm for *Staphylococcus epidermidis*. The results indicate that the ethanolic extract of *Sesamum alatum* leaves has good antimicrobial activity in crude form.

Introduction

Sesamum alatum Thonn. is an erect annual herb belonging to Pedaliaceae family. *Sesamum alatum* grows predominantly as a road-side weed in Tamil Nadu and it spreads by its winged seeds very easily. Even though the young leaves are used for culinary purposes in African countries apart from oil yielding seeds, it is unbothered road-side plant in many parts of Tamil Nadu. Verbascoside and two cyclohexylethanol derivatives, rengyol (2a) and isorengyol (3a), were isolated and identified from aerial parts and *Sesamum alatum* has renal protective and antidiabetic activities (Mariod et al., 2017). Antimicrobial activity of ethanolic leaf extracts of *Sesamum alatum* is scanty, hence the present study has been carried out.

Materials and methods

Based on the preliminary survey made on the distribution of *Sesamum alatum* Thonn. (Pedaliaceae) Salem and adjacent districts Tamil Nadu, along the highways, the plant was predominantly found in Salem-Coimbatore Highways near Perundurai Bye-Pass road (Plate 1). The identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983).

Collection and of leaves

The leaves of *Sesamum alatum* were collected from Salem-Coimbatore Highways near Perundurai Bye-Pass road, Tamil Nadu. The leaves collected were immediately transported to the laboratory of the Department of Botany, Government Arts College, Salem-7 for further processing.

Preparation of crude extract

The leaves of *Sesamum alatum* were washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant was taken in a 250 ml conical flask and added with 100-200 ml of ethanol (95%). After 48 hours, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

Antimicrobial activity

The selected bacterial strains are collected from NCIM (National centre for industrial microorganisms, Chandigarh). The following bacterial species are used for the study. *Micrococcus luteus* (NCIM-2103), *Staphylococcus epidermidis* (NCIM-2493), *Escherichia coli* (NCIM-2065), *Pseudomonas cepacia* (NCIM-2106) and *Bacillus megaterium* (NCIM-2187). Kirby-Bauer disc diffusion technique was used to test the sensitivity of selected test organism to the ethanolic leaf extracts (Bauer et al., 1966). Ciprofloxacin (30 µg/disc) served as control.

The antibacterial activity of plant extracts was determined by resazurin dye reduction method (Karuppusamy and Rajasekaran, 2009). The dye resazurin was obtained from Sigma chemicals and prepared as 10g /l sterile water stock solution, stored in frozen at -20°C, thawed and diluted 1:10 in sterile water when required.

The titreplate resazurin assay was performed in 96-well plates. Two-fold dilutions of each antibiotics and plant extracts were prepared in the test wells in complete nutrient broth, the final antibiotic concentrations being streptomycin 0.06 mg/l and tetracycline 0.12 mg/l. Twenty microlitres of each bacterial suspension was added to 180 µl of antibiotics and plant extracts containing culture medium. Control wells were prepared with culture medium and bacterial suspension only. The plates were sealed and incubated for 12 hr at 37°C. After each incubation time, 5µl of resazurin solution were added per well, colouring them blue. Plates were incubated at 37°C for additional 5 hr. After every

one hour incubation time interval, plates were read for colour change from blue to pink and pink to colour less in live-bacterial strains containing wells. Extracts that showed preliminary microtitre-plate assay were revealed the fast decolouration of resazurin which extracts does not have possessed antibacterial potential. The bioactivity of the extracts were screened by which are all the extracts inhibit the dye reduction.

Inoculums were prepared by various dilutions of (1 × 10G -1 × 10G) growing bacterial strains in Mueller-Hinton broth in 10 ml test tubes. The tubes were sealed and incubated under 37°C for 24h. After the incubation, test tubes were added various concentrations of the plant extracts prepared in the same broth ranges between 0.1 mg/l to 10 mg/l. Positive controls were prepared with only 9 ml of broth containing 1 ml of 0.1% resazurin solution without plant extracts and antibiotics. Antibiotic control tubes were also maintained aliquots of antibiotic solutions with respective bacterial strains in serial concentrations. In each test tubes added 1 ml of 0.1% resazurin solution and the tubes were further incubated at 37°C for 5 hrs.

After the incubation, 1 ml of solution were taken out form each test tube and read the absorbancy (OD) at 590nm in a spectrophotometer for every one hour up to 5 hour. The minimum inhibition concentration (MIC) was defined as the lowest concentration of the extract that prevent colour change in the test tubes as OD is very close to positive control tube.

The resazurin reduction test can be used for colorimetric determination of minimum inhibitory concentration (MIC) of the plant extracts on par with earlier method. After 5 hours of inoculation of sample extracts in different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 µL) with marker dye solution were taken the absorbancy of the cultured broth. The colour changes in the tubes can be markedly visible and also obtained MIC (maximum absorbancy) for potential antibacterial extracts showed the values close to the antibiotic control wells.

Results and discussion

The zone of inhibition (ZI) observed for 1000 mg/ml of ethanol extracts of *Sesamum alatum* leaves in disc diffusion technique was 1.9 cm for *Escherichia coli*, 1.6 for *Pseudomonas cepacia*, 1.4 cm for *Staphylococcus epidermidis* 1.3 cm for *Bacillus megaterium* and 1.0 cm for *Micrococcus luteus*. The ZI for ciprofloxacin was 2.9 cm.

The minimum inhibitory concentration (MIC) was least (62.5 µL) against *Escherichia coli* followed by *Micrococcus luteus* (125 µL), *Pseudomonas cepacia* (250 µL), *Staphylococcus epidermidis* (500 µL) and *Bacillus megaterium* (1000 µL) (Fig. 1).

It is well known fact that different parts of *Sesamum indicum* were demonstrated to exhibit antioxidant, antimicrobial, antiinflammatory, antidiabetic, anticancer, antihyperlipidemic, hepatoprotective, anthelmintic, antileishmanial, gastroprotective, larvicidal, and vasorelaxant activities, among others. Potent pharmacological activities observed *in vivo* particularly highlight the need for further exploration at clinical levels and drug development of identified chemotherapeutic candidates (Amoo et al., 2017).

The methanolic and ethanolic extracts *Sesamum radiatum* have broad spectrum antimicrobial effect against various kinds of micro-organisms (Shittu et al., 2007). The extract of *Sesamum indicum* sprayed with $CuCl_2$ were the most inhibitory against the fungi, *Macrophomina phaseolina* and *Fusarium oxysporum* in case of leaves extract and against *Fusarium oxysporum* in case of stem extract (Syed et al., 2015).

The antibacterial activities of *Sesamum indicum* leaf extracts have been well documented by Ogunsola and Fasola (2014). Like the other species of *Sesamum, S. alatum* in the present study also exhibited antimicrobial activity against selective bacteria. The phytochemical analysis of the ethanolic leaf extract of *Sesamum alatum* may be helpful in the identification of active antimicrobial principle.

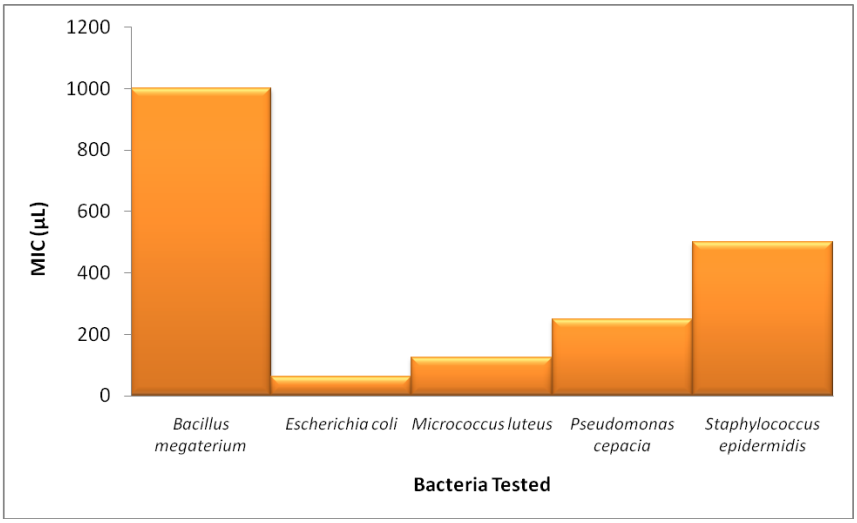

Fig. 1: Minimum Inhibitory Concentration (MIC) of ethanolic leaf extracts of *Sesamum alatum* against different bacteria.

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

Amoo, S.O., Okorogbona, A.O.M., Du Plooy, C.P., Venter, S.L., 2017. *Sesamum indicum*. In: Medicinal Spices and Vegetables from Africa. Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases. Academic Press. pp.549-579.

Bauer, A.W., Kirby, W.M.N., Sherris J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45, 493-496.

Gamble, J.S., Fischer, C.E.C., 1928. Flora of the Presidency of Madras (Vols I-III), Authority of Secretary of State for India in Council, London. 1389p.

Karuppusamy, S., Rajasekaran, K.M., 2009. High throughput antibacterial screening of plant extracts by resazurin redox with special reference to medicinal plants of Western Ghats. Global J. Pharmacol. 3(2), 63-68.

Mariod, A. A., Saeed Mirghani, M.E., Hussein, I., 2017. *Sesamum alatum* (Thonn) Winged-Seed Sesame. In: Unconventional Oilseeds and Oil Sources. Academic Press. pp.39-43.

Matthews, K.M., 1983. The Flora of Tamil Nadu Carnatic, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. 1034p.

Ogunsola, O.K., Fasola, T.R., 2014. The antibacterial activities of *Sesamum indicum* Linn. leaf extracts. Adv. Life Sci. Technol. 18, 28-32.

Shittu, L., Bankole, M.A., Ahmed, T., Bankole, M.N., Shittu, R.K., Saalu, C.L., Ashiru, O.A., 2007. Antibacterial and antifungal activities of essential oils of crude extracts of *Sesame radiatum* against some common pathogenic micro-organisms. Iran. J. Pharmacol. Therapeut. 6, 165-170.

Syed, R.N., Laurentin, H., Splivallo, R., Karlovsky, P., 2015. Antifungal properties of extracts of sesame (*Sesamum indicum*). Int. J. Agric. Biol. 17, 575-581.

How to cite this article:
Sundarakumar, M., Karmegam, N., 2018. Antibacterial activity of ethanol extracts of *Sesamum alatum* Thonn. leaves. Int. J. Curr. Res. Biosci. Plant Biol. 5(3), 38-41.
doi: https://doi.org/10.20546/ijcrbp.2018.503.005