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Antibacterial Activity of Ethanolic Leaf Extract of *Sida rhombifolia* L. Against Gram Negative Bacteria

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Abstract

Different reports on the development of resistance against existing antibacterial agents demand the need for searching new antibacterial compounds. We tested the ethanolic leaf extract of Sida rhombifolia L. (ESR) for antibacterial activity against two gram-negative bacteria named Salmonella typhi and Escherichia coli. The disk diffusion approach was used for this experiment. Results indicated antibacterial action of the extract (500 µg/disk) against these gram-negative bacteria with zone of inhibition about 12mm and 10 mm against Escherichia coli and Salmonella typhi, respectively.

Keywords: Antibacterial resistance, Gram negative bacteria, *Sida rhombifolia*, Disk diffusion method

1. Introduction

Antimicrobial agents are abundant in medicinal plants. A lot of medicinal plants have their own unique medicinal effects against certain microorganisms. Many secondary metabolites of plant, such as tannins, alkaloids, phenolic compounds, and flavonoids have been demonstrated to have antibacterial properties *in vitro* (Manandhar and Dahal, 2019). Antimicrobial Properties such as *Eugenia caryophyllata*, *Cinnamomum verum*, *Myristica fragrans*, *Origanum vulgare*, *Allium cepa*, *Allium sativum*, *Pimpinella anisum*, *Sassafras albidum*, *Morinda citrifolia* etc. Nowadays, because of the global problem of rising microbial resistance to existing antibiotics, search for new antibacterial agents is a crying need. *Sida rhombifolia* L., a perennial of malvaceae family, is native to the tropic and subtropic areas (Echou*et al.*, 1996). In Madagascar and in Cameroon, it is used for the treatment of numerous ailments like diarrhea, cough, ulcer, abscess and furuncle due to its antiseptic, wound-healing potential (Echou *et al.*, 1996; Noumi and Yomi, 2001). Ekramul *et al.*, (2000) mentioned the use of its root and stems to treat piles, heart disease, a fever, and several inflammatory conditions. According to David *et al.*, (1995), methanolic extract of *S. rhombifolia* L. demonstrated anti-tumor and anti-HIV properties. Several researchers have discovered antinociceptive, anti-inflammatory, cytotoxic, antibacterial, and larvicidal properties of organic extracts of *S. rhombifolia* (Venkatesh et al., 1999; Ekramul et al., 2003). In this study, antimicrobial activities of the ethanolic extract have been searched and the results have been discussed here.

2. Methods and materials

2.1 Collection of plant material

Freshly harvested *Sida rhombifolia* L. leaves were cleaned, rinsed, dried, and ground after collection. The taxonomical identification of the plant was done by botanist from National Herbarium Bangladesh and the identification code is DACB 48436.

2.2 Crude extract preparation

250g of powdered plant material were immersed in 1200 ml of ethanol for a few days with occasional stirring. After roughly 10 to 12 days, a thick, viscous, sticky, dark green crude mass (24 g) was visible.

2.3 Chemical and reagents

Analytical grade materials were utilized throughout the study.

2.4 Test organisms

Salmonella typhi and *Escherichia coli* were chosen here were purchased from Bangladesh Council of Scientific and Industrial Research (BCSIR).

2.5 Experimental procedure

A few colonies (3-10) of the organism under study were transferred from the original culture plate into a test tube having 4 ml of tryptose phosphate or trypticase soy broth. These tubes were then incubated for 2 to 5 hours to produce a slightly hazy bacterial dispersion. The solution was then thinned down with distilled water until it matched the density of a standard that was created by combining 0.5 ml of 1% $BaCl_2$ with 99.5 ml of 1% H_2SO_4 (0.36 N). For the sensitivity plates, large 15 cm petri dishes containing Mueller-Hinton agar were used (5 to 6 mm. in depth). Plates were used within four days of preparation and dried for around 30 minutes prior to inoculation. The bacterial broth suspension was uniformly dispersed across the medium's surface in three planes using a cotton swab (not a glass rod or wire loop). The swab was spun against the side of the tube before the plates were seeded to remove any surplus suspension. Test sample disk (ESR 500 µg per disk), blank disk (just solvent), and standard Kanamycin (30 µg per disk) were created. The disks were placed on the agar once the inoculums dried using flamed forceps (3 to 5 minutes) and lightly pressed down to establish contact. The plates were to be incubated for 30 minutes. After an overnight incubatory time, the zone diameters were measured (Bauer *et al.*, 1966).

3. Results

Ethanolic leaf extract of *Sida rhombifolia* L. (ESR) showed inhibition against *Escherichia coli* with zone of inhibition of 12 mm at 500 µg/disk concentration. ESR inhibited *Salmonella typhi* with zone of inhibition of 10 mm at 500 µg/disk concentration. In both cases, we used standard kanamycin 30 µg/disk provided zone of inhibition of 32 mm. The following table (Table-1) displays the diameters of the zone of inhibition against the investigated microorganisms.

Table 1: Comparison of antibacterial activity of ESR with standard Kanamycin

Test organisms	Diameter of zone of inhibition (mm)	
	ESR (500 µg/disk)	Kanamycin (30 µg/disk)
Escherichia coli	12	32
Salmonella typhi	10	32

4. Discussion

Growth of gram negative bacteria *Escherichia coli* was considerably inhibited by ethanolic leaf extract of *Sida rhombifolia* L. Here, this extact causes a zone of inhibition of 12 mm at 500 µg/disk concentration. Salmonella typhi, a gram-negative bacterium, was also inhibited by this extract, with a 10 mm zone of inhibition produced at a concentration of 500 g/disk. Standard aminoglycoside Kanamycin (30 µg/disk) provided zone of inhibition of 32 mm in both cases. Though much prominent antibacterial action was not obtained using this plant extract, isolation of antimicrobial phytochemicals can be led by intensive investigation of this plant.

5. Conclusion

The extract's potential to inhibit gram-negative bacterial strains was evidently demonstrated by the results. According to the findings, gram negative bacteria are significantly sensitive to the leaf extract's antimicrobial effects. Further investigations are required for finding antibacterial activity against more bacterial strains and to explore out important compounds possessing antibacterial activity from this plant extract.

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Conflict of Interest

The writers do not have any competing interests.

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