International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Antibacterial Activity from Aerial Parts of Double Flower Variety of *Tabernemontana Divaricata*

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Abstract: ***Tabernaemontana divaricata is a medicinal shrub which belongs to Apocynaceae family. It acquires many valuable manners with the use of its different parts. Flowers can be used to treat ophthalmic problems, burning sensation and dermatopathic disorders. Leaves and flowers can possess antidiabetic, antioxidant and antibacterial properties. There is no much informations are available related to stem's antibacterial activity. In this study, ethanolic extract of flowers and stems showed more inhibition against Bacillus subtilis, Escherichia coli and Staphylococcus aureus. Ethanolic extract of leaves exposed inhibitory action against Staphylococcus aureus but no inhibition activity against E.coli and B.subtilis.***

Keywords: Tabernaemontana divaricata, Antibacterial activity, Escherichia coli, Bacillus subtilis, Staphylococcus aureus

1. Introduction

Many infectious diseases are the main reason for death of general population (Balagurunathan *et al*., 2011). These illnesses are caused by bacteria and fungi (Akter *et al*., 2012; Hart and Kariuki, 1998; Abebe et al., 2003; Moellering, 2011). Importantly bacteria cause severe infections in human beings and animals. *Staphylococcus aureus* causes food poisoning, local abcesses and superficial skin lesion (Lotifpour *et al*., 2008). 95% of *S.aureus* strains are resistant to antibiotic penicillin around the world (Casal *et al*., 2005).

Escherichia coli mainly affects gastrointestinal tract and cause diarrohea especially in children (Wood CD, 1988; Donnenberg MS, 2002). *Bacillus subtilis* can be responsible for food spoilages including sliminess, production of CO_2 in canned meats and coagulation in milk (N Azu and R Onyeagba, 2006; Frazier, W.C. and Westhoff, D.C., 1991).

Some of the organisms have resistant capacity against few antimicrobial drugs it means that multi-drug resistant microorganisms with lower susceptibility to antibiotics are raised constantly for the treatment of infectious diseases (Dabur *et al*., 2007). So, novel drugs are needed to overcome these problems without side effects. More than 65% of global people use medicinal plants for their health care (WHO, 2001). Likewise 80% of Brazil people utilize only 37% of commercial drugs and almost depends on medicines of natural derivation (Silva NCC et al., 2010). Extracts from plants may serve antimicrobial and antioxidant activity to influence the body system (Ahmad I and Beg A. Z, 2001, muthukumaran *et al*., 2011).

Nature is a place for many plants with different medicinal properties (Neeta patil *et al*., 2013). Humans have used plants for the safer medication of diseases from ancient years. People now changed their life style to use herbal medicine instead of synthetic medicines because of least or no side effects (Kanthlal *et al*., 2011). Medicinal plants have been used as an alternative source of therapeutic drug with the use of its different parts. In different countries, several plants are used for herbal preparations as indigenous system of medicine.

Tabernaemontana divaricata (Family: Apocynaceae) is referred as Crape Jasmine, evergreen shrub or small tree grown in tropical countries. It is otherwise known as Nandiyavattai (Tamil), Nandivriksha (Sanskrit), Nandibatlu (Kannadam), Nandivardhanamu (Telugu), Chandani (Hindi), Kutampale (Malayalam)(C N. Raj, A. Balasubramaniam, 2011). Flowers are cooling, fragrant and milky white in colour used to cure blazing feel, ophthalmitis and dermatopathy. Root can be used for tooth ache and milky latex with coconut oil for head ache. It holds broad range of beneficial activities like anticancer, anti-infection, anti-inflammation, hepatoprotective, astringent and analgesic properties (Bijeshmon P.P and Shibu George, 2014).

2. Literature Surveys

Rahman Md. Ashikur et al., (2011) found that phytochemical analysis and antibacterial activity from the leaves of *T.divaricata* (L) using ethanol, Petroleum ether, diethyl ether, methanol and aqueous extracts. Phytochemical analysis indicated the presence of steroids, saponins, tannins, gums and reducing sugar. All the extracts showed maximum potency against various pathogens like *Staphylococcus aureus, Enterococcus faecalis, Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa*.

Phytochemical screening, physicochemical evaluation and antimicrobial activity were done in leaves of *T.divaricata* using variety of extracts such as petroleum ether, chloroform, methanol, alcohol and water. The presence of all phytochemicals like phenols, flavonoids, steroids, saponins and tannins etc was obtained by the extracts stated above. Chloroform and alcoholic extract showed more inhibition against both species of bacteria which was tested and justified by C N. Raj and A. Balasubramaniam (2011).

Neeta Patil *et al*., (2013) evaluated that antioxidant, antibacterial and cytotoxic activity of *Vitex negundo* L. and *Tabernaemontana divaricata* L. The leaf extracts of both

plants (ethanol, methanol, acetone and water) had antioxidant and antibacterial activity. The cytotoxic activity of the extracts of *V. negundo* L. and *T. divaricata* L. were assessed on THP-1 leukemia cell line using MTT assay and both the plants demonstrated cancer cell inhibitory activity.

Antibacterial activity and powder microscopy of flowers of *T.divaricata* was done and stated by Bijeshmon P.P and Shibu George (2014) using different flower extracts (water, petroleum ether and methanol) were tested by. They found that only the methanolic extract of the flower was effective to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* by disc diffusion method.

It is necessary to identify the antibacterial activity of ethanolic extract from aerial parts of double flower variety of *Tabernaemontana divaricata* against pathogens such as *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*.

3. Materials and Methods

3.1 Extract preparation

Leaves, stems and flowers were collected and washed. It was dried in shade and powdered. Powdered materials were macerated with ethanol using cold percolation method for some days with sporadic shaking. Each mixture was filtered by a piece of clean, white cotton cloth. The filtrates obtained were used for further analysis.

3.2 Microorganisms used for Antibacterial Activity

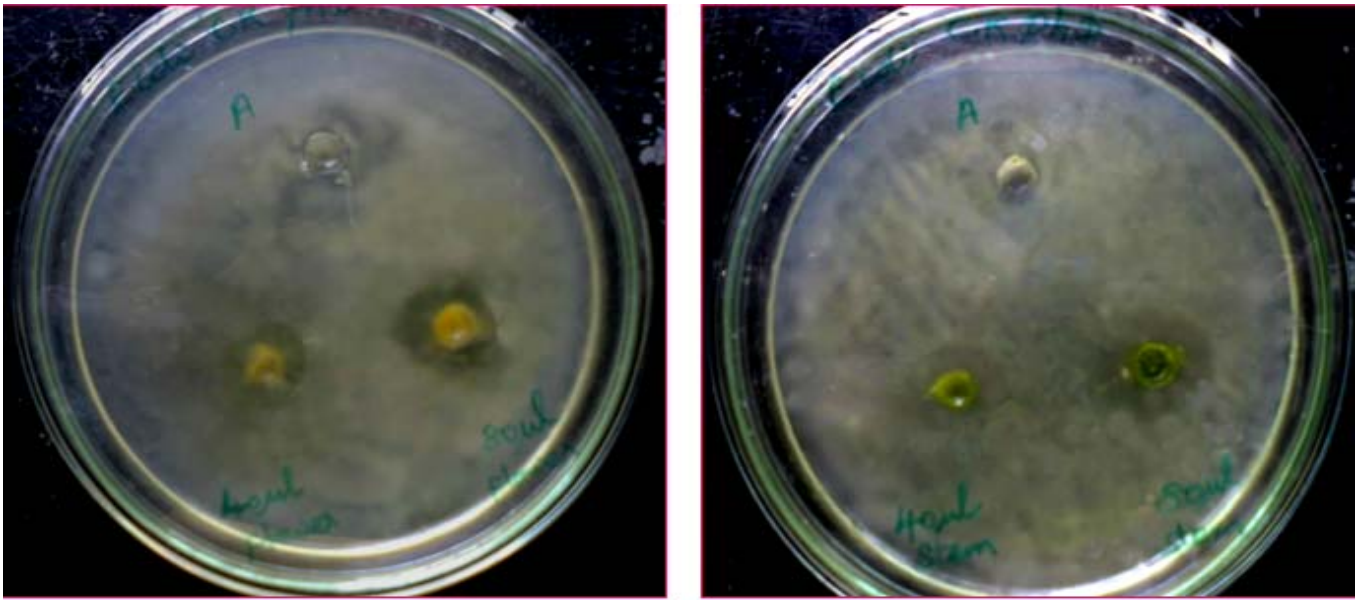
Escherichia coli, Bacillus subtilis and *Staphylococcus aureus*

3.3 Antibacterial Susceptibility Testing

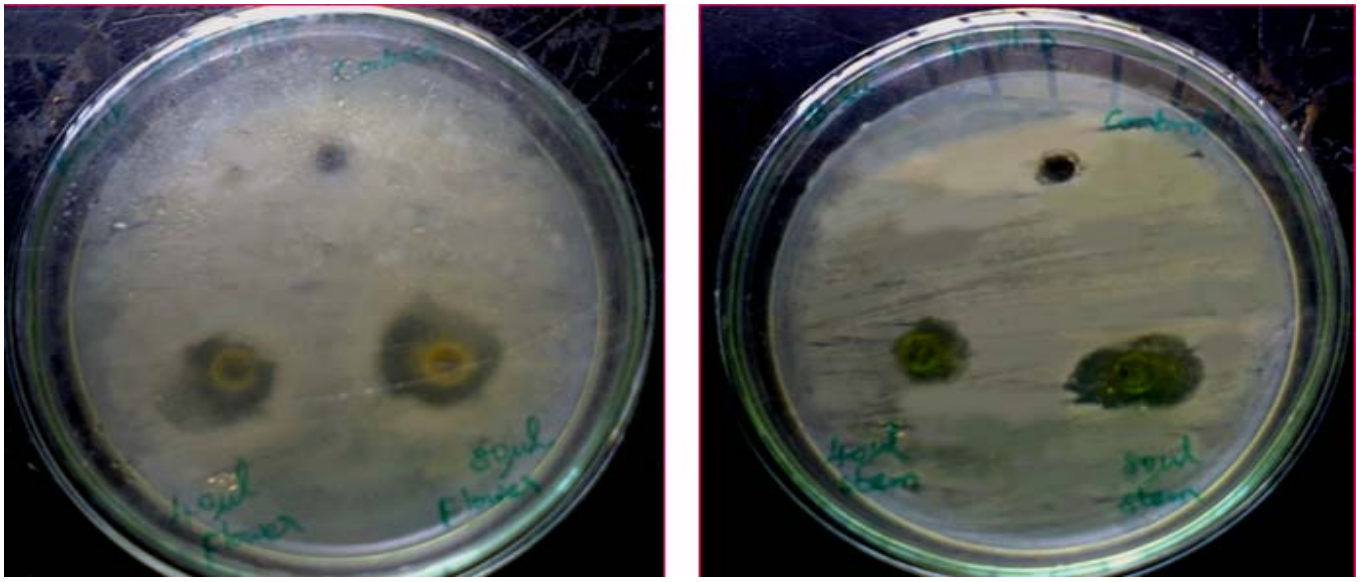
This activity was done using agar well diffusion method. Nutrient agar medium was prepared and sterilized. It was poured into pre sterilized petridishes. Bacterial cultures were spread on the medium using cotton swabs. Three wells could be created. Control (Solvent alone) and two different concentrations of each extracts (40 and 80µl) were kept in the respective wells and marked as B and C. The plates were incubated at 37˚c for 24 hours in room temperature to allow the diffusion of extracts into the medium. Zone of inhibition was observed after 24 hours of inoculation.

4. Results

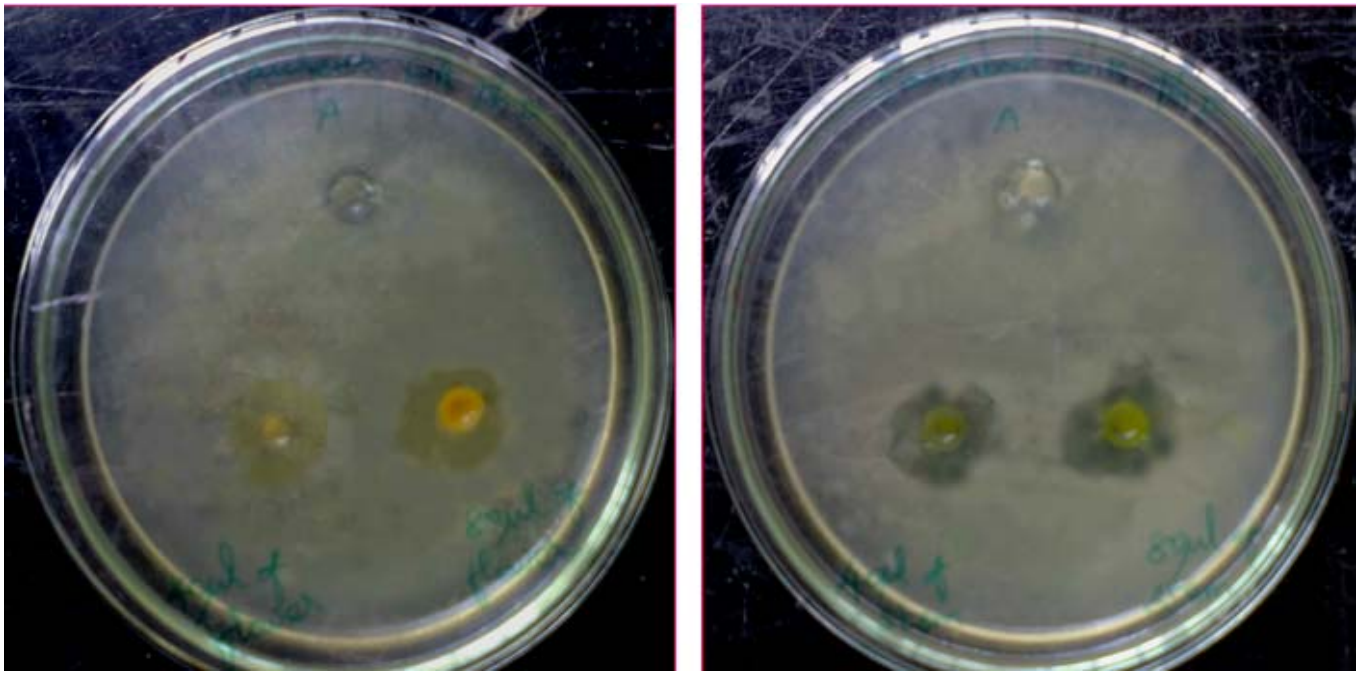
A – Solvent alone, B and C – two different concentrations of extracts (40 and 80µl).



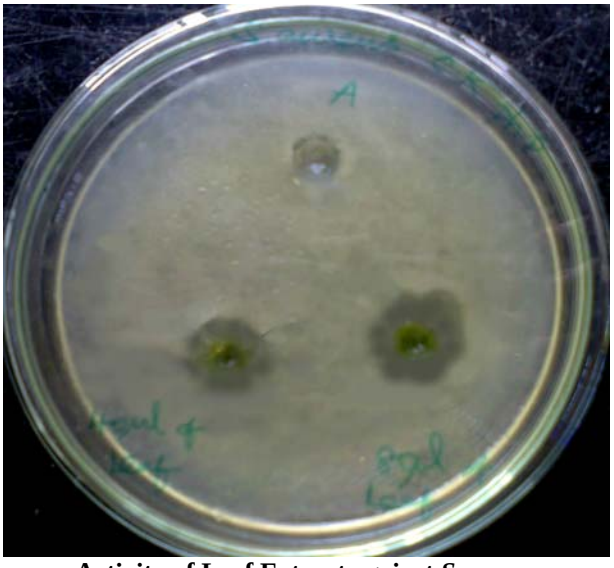
Activity of Flower and Stem Extract against *E.coli*



Activity of Flower and Stem Extract against *B.subtilis*



Activity of Flower and Stem Extract against *S.aureus*



Activity of Leaf Extract against *S.aureus*

Control (A) with solvent alone did not show inhibition zone around the well. But less concentration (40µl) and high concentration of extracts (80µl) revealed low and more inhibition respectively.

5. Discussion

This study investigated that whether the ethanolic extract from aerial parts of double flower variety of *Tabernaemontana divaricata* has capacity to act against pathogens. Ethanolic extract of flowers and stems showed more resistant capacity against *B. Subtilis, E.coli* and *S.aureus* through inhibition zone. Ethanolic extract of leaves had more resistance against *S.aureus* but *E.coli and B.subtilis* exhibited no susceptibility to the leaf extract. If concentration of each extracts increased there is the possibility to observe maximum inhibition against these bacterial species.

Antimicrobial susceptibility test could be used to identify the effectiveness of extracts against bacterial infections caused by pathogens. The plant extracts had bactericidal at higher and bacteriostatic at lower concentrations respectively. 80% of rural people utilized herbal medicines in their life cycle as primary healthcare (Rahman Md. Ashikur *et al*., 2011). In our normal life, antimicrobial substances could be used for bacterial infections at different time intervals (Al-Hussini *et al*., 2014, Urassa, 1997).

Because of costs and side effects of some synthetic drugs, there was a need for other products as natural medicines from plants (Chinnaperumal kamaraj *et al*., 2012).

6. Conclusion

The present study concluded that the ethanolic extract from aerial parts of double flower variety of *T.divaricata* had inhibitory action against *S.aureus.* Stems could be taken for the first time in the study of antibacterial activity. Flowers and stems revealed inhibitory action against *E.coli, B.subtilis and S.aureus*. But no inhibition zone could be found in the leaf extract against *E.coli and B.subtilis*.

7. Future Scope

In future aerial parts of double flower variety of *T.divaricata* can be tested beside microorganisms. Further studies can be done to identify and isolate the compounds present in these parts which are responsible for antibacterial activity such as phytochemical screening and free radical scavenging. To be strong double flower variety of *T.divaricata* must be prove that it can be used as a drug for bacterial infections caused by pathogens.

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