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"INVESTIGATION OF ANTICANCER AND ANTIBACTERIAL ACTIVITY FROM *ACACIA NILOTICA* OF CRUDE AND PURE COMPOUND"

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ABSTRACT

The antimicrobials in the therapeutics of infectious diseases were described over 2,500 years ago. At that time, they were regarded as the solution to all diseases caused by microorganisms. *S. marcescens* has been recognized as the cause of many hospital epidemics and a causative agent of hospitalized infection. It causes several diseases as a secondary infection such as urinary, respiratory, wound and septic arthritis, peritonitis and sinusitis. *S. marcescens* constitutively possesses chromosomally encoded, inducible Ampc β9 lactamases and may acquire plasmid- mediated extended-spectrum β-lactamases (ESBLs). *S. marcescens* was used in a number of classic bacterial transmission experiments, which led to improved understanding of the epidemiology of infection. From 1960 onwards, however, non-pigmented isolates of *S. marcescens* predominated over pigmented strains in the clinical setting and were increasingly implicated in healthcare associated infection. Limited research has been done, and many more pharmaceutical industries are interested in examining their potential as sources of novel medicinal compounds. Many bioactive compounds have been discovered from plants especially from Acacia nilotica, animals and microbes, such as natural products and secondary metabolites, which have been developed into drugs to treat diseases. Historically, natural products in the field of anti-cancer research has made significant achievements, over 60% of the clinical use of anti-cancer drugs originate from plants, marine organisms, microbes, and more than 3,000 species of plants can be used to treat cancer.

KEYWORDS: *S. marcescens,* Acacia nilotica, animals and microbes.

INTRODUCTION

Natural products have been used for centuries for the treatment of several ailments. There are many basic ancient medicinal systems derived from dietary sources. In modern society, economy and technology is more developed, traditional medicines are still used in many countries as basic healthcare. Although many conventional pharmaceutical approaches have been replaced, however there is a current resurgence in the interest in natural products by the general public, and the use of complementary and alternative medicine is increasing rapidly in developed countries. Limited research has been done, and many more pharmaceutical industries are interested in examining their potential as sources of novel medicinal compounds (Zhang *et al.*, 2013). Many bioactive compounds have been discovered from plants, animals and microbes, such as natural products and secondary metabolites, which have been developed into drugs to treat diseases.

Historically, natural products in the field of anti-cancer research has made significant achievements, over 60% of the clinical use of anti-cancer drugs originate from plants, marine organisms, microbes, and more than 3,000 species of plants can be used to treat cancer. Plants are having many potentially active ingredients listed in chemical, biochemical, pharmacological and clinical research data screening. Furthermore, the development of pharmacological experiment technology, the structure of the guide, modification and transformation of natural products have also promoted the rapid development of synthetic drugs, and with the help of modern extraction, modification and transformation, technology is bond to find some new ideas and approaches in the treatment of chronic disease.

Anticancer drugs derived from plants

Natural products derived from fruits, vegetables, herbs and marine products have served us well in combating cancer. The compounds are well characterized as possessing a wide variety of anti-tumor properties for example, induction of apoptosis and autophagy and inhibition of cell proliferation. Active ingredients such as alkaloids, flavonoids, terpenoids, polysaccharide and saponin obtained from medicinal plant have potent biological properties such as anti-tumor, analgesia, anti-inflammatory, immunomodulation, anti-viral, activities. Marine organisms, invertebrates and algae offer rich sources of anti-cancer agents with structurally diverse bioactive compounds and bioactive secondary metabolites that possess various anti-tumor activities; indole alkaloids being the most common. A number of products were first discovered from microbes like antibiotics, which has anti-tumor activity. The anti-tumour activity of most natural anti-neoplastic drugs often do not kill tumor cells directly, but regulates the human immune function to achieve the purpose or both. Division and duplication is a series of important events in cell cycle, and a deregulation of cell cycle can have effect on the development of cancer (Wang and Ren, 2010). DNA topoisomerase I (TopoI) is an essential enzyme involved in cell growth. The inhibition of TopoI is an important anti-cancer pathway. And also, a large number of anti-cancer drugs combat cancer through cell cycle arrest, induction of apoptosis and differentiation as well as through inhibition of cell growth and proliferation, or a combination of two or more of these mechanisms (Abubakar *et al.,* 2012).The search for novel drugs is still a priority target for cancer therapy due to the fact that chemotherapeutic drug resistance of cancers cells.

Acacia nilotica plant

Kingdom : Plantae
Sub- kingdom : Tracheobionta
Super division : Spermatophyta
Division : Magnoliophyta
Sub class : Rosidae
Order : Fabales
Family : Fabaceae
Genus : *Acacia*
Species : *Acacia nilotica.*

Numerous studies in *Acacia nilotica* showed interesting biological activities (Lee *et al*., 2011). It is observed that many newly synthesized drugs originate from natural plant products. All parts of the medicinal plants are useful for pharmaceutical purpose. Various parts of the plants are collected by local and folk communities all over the world for their use but these are generally collected in low quantities. A number of chemical components are present in medicinal plants which can be utilized for the treatment of infectious as well as chronic diseases. These unique therapeutically agents are screened repeatedly by clinical microbiologist. These chemically active agents are secondary metabolites which show antimicrobial activity and are usually a combination of different constituents. Hemamalini *et al.* (2013) explained that *A. nilotica* is known as worst weeds because of its impact on environment, invasiveness and unlimited potential for spreading in the plant community. Now a days, recent experiment and studies suggest that this weed possesses some efficient chemicals which help to cure human diseases. Previous research conducted on the plant extract has shown antimalarial, antibiotic, antibacterial, anthelmintic, antifungal, anti-diarrhea, antioxidant, anti-denaturation, molluscidal and anti-cancer activity.

Hence, the main objective of this study was to exploit the hidden potential of *A. nilotica*. Approximately seven phytochemicals were identified namely from this plant like Hydroxy citronellal, 3-picoline-2-nitro, Lavandulyl acetate, 1-acetyl beta carboline, Propionic acid-2-chloro, Trans decalone, D-Glucoronic acid and ethyl ester by GC-MS and each compound was evaluated biologically and most of them were found to be potent. Photochemical analysis of the aerial parts of *A. nilotica* demonstrated the presence of polyphenolic compounds and flavonoids in the flowers. Tannins, volatile oils, glycosides, coumarins, carbohydrates and organic acids are reported in the fruits (Shanawany *et al.*, 1996). Babul has been reported to contain l-arabinose, catechol, galactan, galactoaraban, galactose, N-acetyldjenkolic acid, sulphoxides pentosan, saponin and tannin. Seeds contain crude protein 18.6%, ether extract 4.4%, fiber 10.1%, nitrogen-free extract 61.2%, ash 5.7%, silica 0.44%, phosphorus 0.29% and calcium 0.90% of DM.

Serratia marcescens is a motile, short, rod-shaped, Gram-negative, facultative anaerobic bacterium, classified as an opportunistic pathogen. *Serratia marcescens* was first thought to be harmless (non-pathogenic). *Serratia marcescens* is ability to produce red pigmentation; it was first used in 1906 as a marker in order to trace bacterial activity or transmission (Cappuccino *et al.*, 2005). Currently 14 species of *Serratia* are recognized within the genus, eight of which are associated with human infection. Species implicated inclinical infection *S. marcescens*; *S. liquefaciens* an *S. odorifera* are best known (Mahlen *et al.*, 2011). Most of the *Serratia* sp. clinically isolated and large number of strains serve as human pathogen. *S. marcescens* is credited with a long fanatical history dating back to antiquity, its ability to produce a red pigment it was described as having ‘masqueraded’ as blood.

Acacia nilotica (L) Del. is a medicinal plant belonging to the family Mimosaceae. It is a medium size tree locally named as “Babul” or “Kikar” and is widely distributed in tropical and subtrop-ical regions. In traditional medicine, it is believed that *A. nilotica* is rich in nutrients and contains therapeutic compounds that are capable of prevention, mitigation and treatment of various infectious diseases and pathological conditions. Ayurvedic medicine practices suggest the use of leaves, bark and pods of *A. nilotica* against cancer, cough, diarrhea, fever, small pox, piles and menstrual problems. The plant is rich in polyphenolic compounds, in which catechins are hypothesized to possess antiox-idant and anti-inflammatory activities. The plant *A. nilotica* is reported to have antibacterial effects against pathogenic microorganisms such as *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Plant extracts containing high amounts of bioactive compounds especially antioxidants, have the potential of being used in food, agriculture, nutraceuticals, cosmetics and pharmaceutical products. The plant phytochemicals and their products are generally nontoxic and contain many medicinal benefits. Antioxidant activities of plants are commonly correlated with their polyphenolic compounds content (Singh and Kumari, 2015). Plants also contain secondary metabolites in addition to minerals and primary metabolites that are responsible for antioxidant and antibacterial potentials. The use of such secondary metabolites alone or in combination with antibiotics against drug resistant pathogens can be an alternative approach to overcome the escalating issues of drug resistant infections. The present study is to investigate the *in vitro* antimicrobial activity of *A. nilotica* plant leaf acetone crude and previously acetone extract purified compound pyrogallol against *S. marcescens.*

MATERIALS AND METHODS

Plant Material

The fresh leaves of *A. nilotica* were collected from the Foundation for Revitalization of Local Health Traditions, Bangalore, India (Latitude 12.9715987; Longitude 77.5945627).

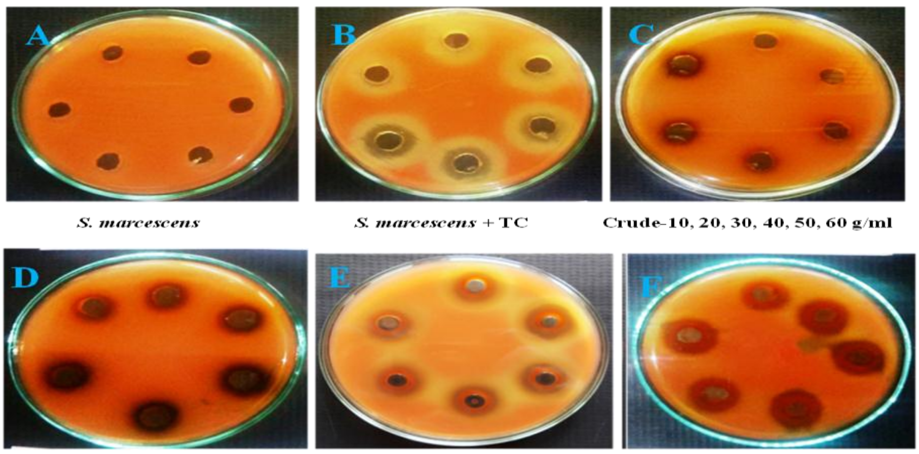
RESULTS

In vitro anti *S. marcescens* activity of crude and purified compound

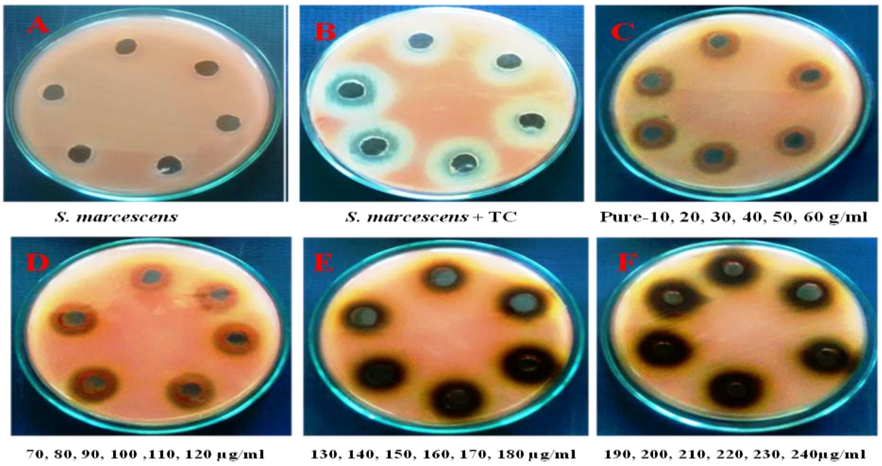
Acetone extract of *A. nilotica* and pyrogallol were tested against *S. marcescens.* DMSO is used as control since the extract was diluted. Solvent has no effect on the growth of bacteria. Tetracycline is used as the positive control.

Minimum inhibitory concentration value of crude extract; anti *Serratia marcescens* agents standard antibiotics and negative control was used as positive controls in this investigation. The rest of the crude extract also exhibited high activity than the standard antibiotics used in this study. The finding of present study revealed that *A. nilotica L.;* contain potent antimicrobial property against *S. marcescens.* The results obtained from the agar well diffusion assay regarding the MIC range of the tested bacteria were shown in (Fig 5: antimicrobial activity of acetone crude extracts *Acacia nilotica* against *S. marcescens*). Based on the inhibition zone for the tested organism the minimum zone of inhibition was observed 70µg/ml in the acetone extract. Fig 6: antimicrobial activity of pyrogallol against *S. marcescens*). Based on the inhibition zone for the tested organism the minimum zone of inhibition was observed 60µg/ml in the pyrogallol. Outcome showed that sample, pyrogallol with organic extracts having least MIC range which means it posses the most significant antimicrobial activity against *S. marcescens.*

In the study, Acetone extract of *A. nilotica* and pyrogallol was subjected to its bactericidal activity against *S. marcescens*. Leaves extracts were subjected to agar well diffusion assay against Gram-negative bacteria. Pyrogallol inhibited the growth of the tested bacteria and MIC results were summarized in **(Fig. 4).** Based on the inhibition zone for the tested organism the minimum zone of inhibition was observed 60µg/ml in the pyrogallol activity better than the a minimum zone of inhibition was observed in crude extract MIC stared with Gram negative bacteria *Serratia marcescens* 70µg/ml, This is due to the active biomolecule present in the acetone extraction of *A. nilotica* and pyrogallol crude extract such as tannins, sterols, enzymes, protein, sugars, alkaloids, acids, terpenes, and polyphenolics. The results observed that plant acetone leaf extract and purified compound showed prominent bactericidal activity.



70, 80, 90, 100 ,110, 120 µg/ml 130, 140, 150, 160, 170, 180 µg/ml 190, 200, 210, 220, 230, 240µg/ml



To determine the cell viability of *S. marcescens* by using Flow cytometry analysis.

FCM analysis of bacteria stained with this kit typically allows differentiation of cell populations into a profile of intact/viable or permeabilized/damaged. Therefore, changes in bacterial membrane permeability of *S. marcescens* treated with *A. nilotica* extracts displaying antimicrobial properties were evaluated at the range of concentrations cells ($1x10^5$ cells per ml) using the annexin V-FITC and propodium iodide double staining FCM analysis. Sample representative results of FCM analysis are presented with percentage of populations marked within each region (Figure8). The fluorescence profiles obtained were based on reference to two control populations of untreated cells indicative of intact/live cells and the other of plant-treated cells indicative of permeabilized/damaged cells.

In the upper panel of (Figure 8a) indicates without treatment acetone extract of *A. nilotica* of crude, results showed control population *S. marcescens* of live cells 99.90% were appeared. Where incase of (Figure 8b) indicates slightly increased damage of cells by induced plant extract of crude treatment after 1 hour, results showed clear shift the cells from live cells to dead cells (47%). (Figure 8c) indicates after 2 hrs increased dead cells (57%), (Figure 8d) indicates after 3 hrs more number of dead cells (61%) wear appeared.

In the upper panel of (Figure 8e) indicates without treatment purified compound of pyrogallol of *A. nilotica*, results showed control population *S. marcescens* of live cells 99.90% were appeared. Where incase of (Figure 8f) indicates slightly increased damage of cells by induced plant extract of crude treatment after 1 hour, results showed clear shift the cells from live cells to dead cells (34%). (Figure 8g) indicates after 2 hrs increased dead cells (42%), (Figure 8h) indicates after 3 hrs more number of dead cells (71%) wear appeared.

From the values of *S. marcescens* viability reduction from 99.90% to 39% with crude extracts and *S. marcescens* viability reduction from 99.90% to 29% with pyrogallol. In addition, the percentage of live cells of *Serratia marcescens* was highly reduced treated with leaf part of *A. nilotica* pyrogallol compare than crude extracts treatment.

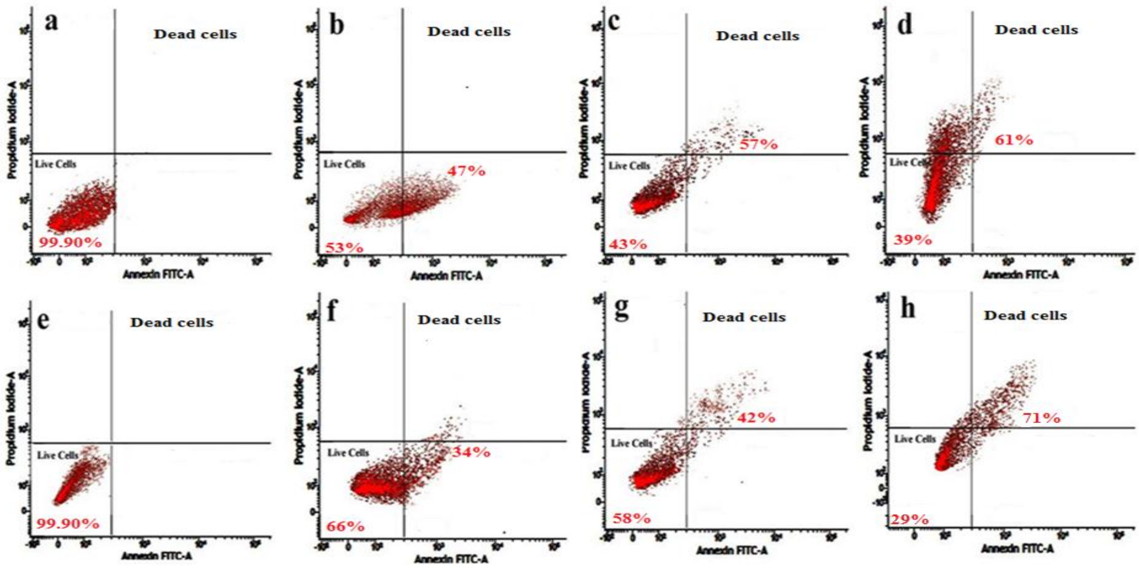


Figure 8: Flow cytometry dot plots of annexin V-FITC and propodium iodide double staining on *Serratia marcescens* (MTCC 4822) treated with acetone extract of *A. nilotica* crude and pure compound pyrogallol; In upper Panel (a) untreated cells (control), (b)1hr treated of crude extract, (c) 2hr treated of crude extract, (d) 3hr treated of crude extract; In lower panel (e) untreated cells (control), (f) 1hr treated of pyrogallol, (g) 2hr treated of pyrogallol, (h) 3hr treated of pyrogallol.

DISCUSSION

Numerous studies on *A. nilotica* showed various interesting biological activities. The findings of the present study revealed that *A. nilotica* contain potent antimicrobial property against tested *S. marcescens*. Outcome shows that *in vitro* sample, acetone extract of *A. nilotica* having MIC range which means it posses the most significant antimicrobial activity against microbial strains following bark and leaves extract. Earlier, a few researches have been done on the account of comparative antimicrobial activities of this plant. However, many report available on the relative study of *in vivo* and *in vitro* samples. These observations are similar and more antibacterial effective compared, the forty-five species of 29 plant families used in the traditional medicine by Iranian people, showed antibacterial activities against *Serratia marcescens* and more of the bacterial species. From the 29 plant family only *Peganum* *harmala, Ziziphus spini-christi, Ziziphus spini-christi* showed low activity against *Serratia marcescens* (15 mm) (Shahidi Bonjar, 2004). The in vitro antibacterial activity of the essential oil isolated from *Cinnamomum zeylanicum* bark, *Syzygium aromaticum* flowers, against Gram-negative. Clove oil showed significant inhibitory effect against *Staphyllococcus aureus* (20 mm), *Streptococcus pyogenes* (23 mm) and *Escherichia coli* (25 mm), *Pseudomonas aeruginosa* (16 mm), *Proteus mirabilis* (23 mm), *Serratia marcescens* (20 mm), *Enterobacter cloacae* (25 mm), *Klebsiella pneumonia* (20 mm).

Gangoue *et al., (*2009) in his study in vitro antimicrobial activity of Stem bark of *Bridelia micrantha* and leaves of *Dortenia picta* against beta–lactam resistant bacteria. The study was initiated to evaluate the antibacterial activity of 17 crude extracts from 12 medicinal plants against the beta-lactame resistant *bacteria (Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Serratia marcescens, Acinobacter baumannii, Staphylococcus aureus* and *Enterococcus* species) by using disc diffusion and agar dilution assay. The crude plant extracts demonstrated broad spectrum activity against all bacteria tested with inhibition zones with range of (8 to 30 mm). Majowicz 2010 classified and scheduled different plants according their antimicrobial activity and which part of the plant was active. He also explained that the importance of using plant extracts instead of antibiotics as antimicrobial agents. Many published report showed the effectiveness of traditional herbs against microorganism, as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al.*, 2002). In this regard, plants have given western pharmaceutically important compounds and a number of top-selling, drugs of modern time, e.g. quinine, artemisinin, taxol, camptothecin, (Tshibangu *et al.*, 2002). Acquired resistance to antibiotics in bacteria over 25 years now constitutes a serious threat to public health (WHO publication 2001).The phenomenon is further compounded by demographic factors (population growth and urbanization) which generate fertile conditions for the transmission of infections and new opportunities for inter-species traffic of pathogens to man. Problem of antibiotic resistance in both hospital-acquired (nosocomial) and community virtually obsolete.

According to the World Health Organization (WHO), medicinal plants would be the best source for obtaining variety of drugs (Nair, 2006). These evidences contribute to support and quantify the importance of screening natural products. From ancient times, different parts of medicinal plants have been used to cure specific ailments. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants (Farombi *et al.*, 2003). Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. Therefore, on the basis of this, the present study was intended to screening the plant *A. nilotica* L. for the significant activities of antimicrobial activities and water extracts of *Acacia nilotica*, *Justicia* zelanica, *Lantana cameraman, Saraca asoca* exhibited good activity against all the bacteria tested and the MIC was recorded.

From the studies, it is concluded that the traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. From the above results and discussion it can be concluded that the *Acacia nilotica L.* exhibited the potent antimicrobial and anticancer substances. In this study, sensitivity of *S. marcescens* to extracts of *A. nilotica* crude and pyrogallol was obvious. Hence, the risk of infections induced by these *S. marcescens* can be reduced and treatment of these infections can be increased through using *A. nilotica* extracts. According to the current study and assessments of *A. nilotica* effects, it is recommended to develop investigations on synergistic effects of this plant with drugs effective on *S. marcescens* and other pathogenic Gram negative bacteria. Finally, it is recommended to use *A. nilotica* extract in producing new antibiotics. There is need for developing drugs from plants as microorganisms are becoming resistant to antibiotics thereby creating health problems. In fact these herbs are very useful in pharmaceutical industries.

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