ANTIMICROBIAL ACTIVITY OF ANDROGRAPHIS PANICULATA FLOWER EXTRACTS

ABSTRACT

The present study describes the phytochemical profile and antimicrobial activity of *Andrographis paniculata*. For the present investigation, samples of *A. paniculata* extracts, obtained by extraction in methanol, respectively, were used for their antimicrobial activity. The antibacterial activities were assessed by measuring the diameter of the inhibition zones, MIC and MBC values. This is the first report on analysis of antimicrobial components from *A. paniculata*, and our results confer the utility of this plant extract in developing a novel broad spectrum antimicrobial agent. Antimicrobial activity of leaf extract of Andrographispaniculata was studied using different solvent like chloroform, acetone, ethanol and water against bacterial strains like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus sp.*, *Micrococcus luteus*, *Bacillus sp.* and two strains of fungi which are *Saccharomyces cerevisae* and *Aspergillus niger*. The antimicrobial activity was determined by disc diffusion method. Out of the four extract used, acetone and ethanol extracts were found to be highly active against *Staphylococcus aureus* and *Bacillus subtilis*.

KEYWORDS: ANDROGRAPHIS PANICULATA FLOWER, ANTIMICROBIAL ACTIVITY

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INTRODUCTION

A. paniculata is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The therapeutic value of Kalmegh is due to its mechanism of action which is perhaps by enzyme induction. The plant extracts exhibits anti typhoid and antifungal activities. Kalmegh is also reported to possess antihepatotoxic, antibiotic, antimalarial, antihepatitic, antithrombogenic, antiinflammatory, anti-snake venom, and antipyretic properties to mention a few, besides its general use as an immunostimulant agent.

Andrographolide, the chief constituent extracted from the leaves of the plant, is a bitter water-soluble lactone exhibiting protective effects in carbon tetrachloride induced hepatotoxicity in rats. Its LD$_{50}$ in male mice was 11.46gm/kg, ip. This bitter principle was isolated in pure form by Gorter (1911). Such other activities as liver protection under various experimental conditions of treatment with galactosamine, paracetamol etc. are also attributed to Andrographolide. The hepato protective action of andrographolide is related to activity of certain metabolic enzymes. (Kishore PH et al., 2003.)

Andrographis paniculata plant extract is known to possess a variety of pharmacological activities. Andrographolide, the major constituent of the extract, is implicated in its pharmacological activity. A study has been conducted on the cellular processes and targets modulated by andrographolide treatment in human cancer and immune cells. Andrographolide treatment inhibited the in vitro proliferation of different tumor cell lines, representing various types of cancers. The compound exerted direct anticancer activity on cancer cells by cell cycle arrest at G0/G1 phase through induction of cell cycle inhibitory protein p27 and decreased expression of cyclin dependent kinase 4 (CDK4). (Reddy MVB et al., 2003.) Immuno stimulatory activity of andrographolide is evidenced by increased proliferation of lymphocytes and production of interleukin 2. Andrographolide also enhanced the tumor necrosis factor α production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity. The in vivo anticancer activity of the compound is further substantiated against B16F0 melanomasyngenic and HT 29 xenograft models. These results suggest that andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential for being developed as a cancer therapeutic agent. (Rao YK et al., 2004.)

The herb is the well-known drug Kalmegh ‘green cheetah’, and forms the principal ingredient of a household medicine (‘alui’), used as a bitter tonic and febrifuge. The Tamils have been using Nilavempu - as it is called in Tamil - for centuries. In Siddha medicine, Andrographis Paniculata is used widely to treat fevers like chikenguinea, swine-flu, typhoid etc.
MATERIALS AND METHODS:

Preparation of microbial culture

Medium preparation

Nutrient agar was used to culture microbes used for antimicrobial susceptibility test. Nutrient agar was one of synthetic medium used for culturing non-fastidious microorganisms. Most bacteria can grow on the surface of the agar to produce small colonies. In order to make nutrient agar, 20 g of nutrient agar was dissolved in 1 liter of distilled water. The solution was sterilized using autoclave at 121°C for 10 minutes. The melted agar was poured into sterile petri dish immediately after it was taken out from the autoclave to prevent it from hardened. The agar was let cool and hardened in the petri dish. The petri dish was set upside down to prevent formation of water droplets that will disrupt the growth of microorganism and stored at temperature of 3°C. For the growth of *Escherichia coli*, specific medium was used which was Luria-Bertani agar and in order to prepare 1 liter of agar, 10g of tryptone, 5g of yeast extract, 10g of sodium chloride and 15g of agar-agar powder were dissolved in 1 liter of distilled water. Moreover, there were also specific medium to grow fungi which were on Potato Dextrose agar (PDA) This agar was prepared based on the product manufacturer instructions by dissolving 39 g in 1 liter of distilled water and sterilized in an autoclaved at 121°C for 10 minutes before used.

Microbial culture

In order to study for the antimicrobial effects of the extracts, there were eight groups of microorganism select to be tested. The microorganisms used in this study are *Staphylococcus aurous*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus sp.*, *Micrococcus luteus*, *Bacillus sp.*, and two strains of fungi which are *Saccharomyces cerevisae* and *Aspergillus niger*. The bacteria were re-identified using several methods for bacterial identifications.
Culturing microorganisms on growth media

After preparing the growth media, all strains of microorganisms were cultured on to the agar plate and the broth. The cultures were left overnight in an incubator at 37°C for the microorganisms to grow. As for the fungi, they were culture on PDA agar plate and the plate was left for 5 days for the fungi to grow and form spores.

Identification of the bacteria

(a) Gram staining method

Gram staining method is a method used to identify the morphology of the bacteria by using dye to react with the specific cell structures so that these structure may be visible for example flagella, endospores and cytoplasmic inclusions. The most widely used stain for bacterial identification is the gram stain. By using gram stain, the bacteria can be divided into two large groups that are gram positive and gram negative. The different are based on structure of the cell wall where the gram positive strain will stain blue-purple and the gram negative will stain pink-red.

The method starts by preparing bacterial smear before applying the dyes. The solid culture was transferred onto the glass slide and mix with a drop of water to dilute it. After that, the smear was allowed to air dry and followed by heat fix it using Bunsen burner several time. However, the glass slide must not be close to fire as it might become hot and break. After the smear is ready, it was then flooded with crystal violet and the stain was let stay for 30 seconds. The stain was then wash off using distilled water. Next, the stain was flooded with iodine solution for 10 seconds and drain off excess stain using distilled water. Decolorisation of the stain took place by using alcohol and it was then washed off using distilled water. Finally, the smear was counterstain using safranin for 30 seconds and wash with water. The smear was dry by heating. When finished, the glass slide was examined under oil immersion objective.

Antimicrobial susceptibility tests

Preparation of Mueller-Hinton agar

Mueller Hinton agar is a growth medium used for antimicrobial susceptibility test by disk diffusion method. The protein free medium have been developed by Mueller and Hinton in 1941 to isolated pathogenic strains Neisseria The agar are usually appear as translucent and light amber in colour. Mueller-Hinton agar was prepared according to the manufacturer suggestion. 34g of the Mueller-Hinton agar powder was weight and dissolved in one liter of demineralized water. In this case, the deionized water was used because it was found to be similar to demineralized water. The solution was then sterilized by autoclaving at 121°C for 18 minutes then pour onto the petri dish. The agar was let cool and kept at room temperature for one day to seek for any contamination.

Preparation of saline solution

Antimicrobial susceptibility test requires 0.85 % to 0.9 % saline solution for the dilution of microbial culture before applying onto the plate containing to adjust the turbidity. Saline solution was prepared by using 4.25 g of sodium chloride and dissolved in 500 ml of distilled water then autoclaved at 121°C for 18 minutes to sterilize the solution.
Inoculating microorganisms on Mueller Hinton agar
Each bacterial culture was streaked onto nutrient agar to obtain single colonies and incubate overnight at 37°C. After incubation, one or two single colonies and inoculate in 0.85% saline solution and adjusted the turbidity to meet the 0.5 McFarland turbidity standards. The standard is based on the measurement for the absorbance at wavelength 620 to 625 nm and the turbidity must be around 0.08 to 1 (Basri and Fan, 2005). If the absorbance increase, the addition of more saline solution is required while addition of more bacterial colonies can increase the absorbance. Next, sterile cotton swab was used to inoculate the bacterial suspension on Mueller Hinton agar. The cotton swab must be pressed firmly against the wall of the tube to avoid taking too much colonies and remove excess fluid. By using the cotton swab, the bacterial colonies was streaked onto the surface of the agar three times in the different directions by rotating the plate each time to ensure that the bacterial distribute evenly on the agar. In addition, around the agar should also be swab with bacterial colonies.

Preparations and application of antimicrobial discs
The prepared extracts was diluted to five different concentrations of 5, 10, 15, 20 and 25 μg/μl and sterile filtered using 0.2 μm membrane filter. After preparing the extracts, it was applied onto 5 mm diameter sterile disc obtain from Whatman filter paper No. 1. The disc containing the extracts was impregnated on the surface of the agar within 15 minutes after bacterial inoculum. The discs were placed individually on the agar using sterile forceps gently. There were six discs on the agar with distances and the plate was duplicated for each bacterial strains. Not more than twelve discs can be applied onto the agar surface to avoid an overlapping of the inhibition zone by the extracts. In this study, there were three control used which were disc containing solvent, 80% ethanol and disc containing distilled water as the negative control whereas disc containing commercially prepared antibiotic chloramphenicol 30μg/μl as the positive control. For antifungal, the spores of the fungi were applied on PDA with the impregnated discs added onto it. Plate containing extracts impregnated with the discs were incubating for 24 hours at 37°C for bacteria and 30°C for 7 to 14 days for fungi. The antibacterial and antifungal activities were measured by the inhibition zone.

Recording data and interpreting the results
The results were collected after 24 hours of incubation period and the inhibition was measured using ruler in millimeter. This was then compared to the standards in the literature review. An inhibition zone less than 6 mm was not applicable. Data was then presented in the form of table.

RESULTS:
The methanolic and aqueous extracts of *A. paniculata* were assessed at 3 different concentrations by using disc diffusion method against 10 bacterial strains notable for causing chronic skin infections and expressed as the average diameter of the zone of inhibition of bacterial growth around the disc. The MIC and the MBC of active extracts were determined by the agar dilution and micro broth dilution assays respectively. The extracts displayed relative antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 6.00 ± 1.00 to 12 ± 0.76mm.
The Gram-positive strains used for this study were the most susceptible to growth inhibition by the plant extracts forming zones of inhibition ranging from 7.00 ± 0.00 to 12± 0.76mm. The DCM extract was found to exhibit the least potent antibacterial activity against
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