EVALUATION OF *ANDROGRAPHIS PANICULATA* (BURMF) WALL EX NEES, LEAF EXTRACTS AND ANDROGRAPHOLIDE ON GASTROINTESTINAL PATHOGENS

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ABSTRACT

Andrographis paniculata (BurmF) Wall Ex Nees called as Kalmegh or "King of Bitters" is used in southeast Asia for treating several ailments. The aim of this study was to determine the antibacterial activity of *Andrographis paniculata* leaf extracts. The agar diffusion and micro-dilution methods were used to determine the antibacterial activity of the Petroleum ether and chloroform extracts. Chloroform and petroleum ether extracts inhibited *Shigella*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Salmonella paratyphi A* at concentration of 10 and 1mg/ml respectively. The antibacterial property exhibited by the extracts were due to the presence of Andrographolide which was confirmed by HPLC analysis. Standard Andrographolide inhibited all bacterium except *E.coli* which indicates that it is a broad spectrum antibacterial compound. Although Andrographolide was effective against various gastrointestinal pathogens it does not have any harmful effect on the native flora.

Keywords: Antibacterial activity, *Andrographis paniculata*, Andrographolide.

INTRODUCTION

In the recent years, focus on the use of traditional medicine to treat human ailments has been revived all over the world. Medicinal plants used in traditional systems possess immense potentials. *Andrographis paniculata* (Burm.F) Wall Ex Nees (AP) also called Kalmegh or "King of Bitters" belongs to family Acanthaceae. It has been used for centuries in Asia to treat gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic infectious diseases. The Indian Pharmacopoeia literature indicates that it is a predominant constituent of at least 26 Ayurvedic formulations¹. The entire herb possesses various activities like anthelmintic, alterative and tonic, hence used in treatment of dysentery, cholera, diabetes, influenza, bronchitis, swelling and itches². The plant is also reported to possess hepatoprotectant and antioxidant properties³. A recent study has revealed that *Andrographis paniculata* has a broad spectrum of pharmacological effects such as anti-inflammatory⁴, antiviral⁵, anti-malarial⁶, anticancer⁷⁻⁸ and immune stimulatory effects⁹. The plant contains many important secondary metabolites such as diterpenoids. A number of diterpenoids and diterpenoid glycosides of similar carbon skeleton have been isolated from *Andrographis*, mainly the bitterest compounds. The leaves of *Andrographis paniculata* contain the highest amount of Andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest¹⁰. Andrographolide has been isolated in pure form and it has shown various pharmacological activities like anti-platelet aggregation, anti-hyperglycemic, analgesic, antithrombotic, thrombolytic, antipyretic¹¹ and antibacterial properties¹². This study attempts to investigate the antibacterial effect of the petroleum ether, chloroform leaf extract and the standard Andrographolide on gastrointestinal pathogens like *Shigella* Sp., *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella paratyphi A* and *E.coli*.

MATERIALS AND METHODS

Extraction of Plant Material

Healthy leaves of *Andrographis paniculata* were selected and dried. The dried leaves were ground and crushed into coarse powder. About 50g of leaf powder was soaked in solvents such as chloroform and petroleum ether with twice its volume i.e (1:2) separately. The plant material was extracted thrice with the respective solvents and concentrated in a soxhlet apparatus.

Antibacterial Assay

Anti-bacterial screening was performed using agar diffusion assay. The crude extracts were dissolved in 10 µl of DMSO and concentrations of 2, 4, 6, 8, 10 mg/ml of chloroform extract and 0.2, 0.4, 0.6, 0.8, 1.0mg/ml of petroleum ether extract was prepared. Approximately 100µl of different concentration of the extract were loaded into the respective wells and incubated for 24 hrs at 37°C. Standard Andrographolide obtained from Dr. Pundarikakshudu, Avance phytotherapies, Ahmedabad, India; was used as standard and zone of inhibition was recorded.

Minimum Inhibitory Concentration

Minimum inhibitory concentration of the extracts were determined by dilution broth method in a 96 well microtitre plate concentration ranging from 5.5mg-6.4mg/ml and 0.55mg-0.64mg/ml for chloroform & petroleum ether extracts that were diluted with nutrient broth. The bacterium was inoculated and incubated at 37 °C for 24 hrs. The growth of the bacterial culture was assessed by determining the optical density at 540nm.

HPLC Analysis

High performance liquid chromatography was performed to confirm the presence of Andrographolide in the petroleum ether extract. The chromatography was performed on C18 Shimadzu column (4.6x20mm). The metabolite was dissolved in methanol and 20µl was injected into the column. Mobile phase used was methanol with a flow rate of 1ml/min

and absorbance was determined at 245nm using UV PDA detector.

Figure 1: Effect of petroleum ether leaf extract of *Andrographis paniculata* on the bacterium. All values in triplicates

Figure 2: Effect of chloroform leaf extract of *Andrographis paniculata* on the bacterium. All values in triplicates

Figure 3: Effect of Andrographolide (100µg/ml) on bacterium. All values in triplicates. S-Streptomycin (0.1mg/ml)

Figure 4: HPLC analysis of petroleum ether extract of *A. paniculata* with standard Andrographolide

RESULTS

Screening for antibacterial activity of leaf extract of *Andrographis paniculata* revealed that petroleum ether extract inhibited *Shigella Sp.*, *Proteus vulgaris*, *Klebsiella pneumoniae* with maximum inhibition zones being 5mm at 1mg/ml respectively. However, *Salmonella paratyphi A* was inhibited with negligible difference in inhibition when compared to the other forms of bacteria. *Klebsiella pneumoniae* & *Salmonella paratyphi A* were not inhibited at a concentration of 1mg/ml of chloroform extract. However at 10mg/ml, these two bacterial forms were inhibited maximum with inhibition zone of 6 & 7mm respectively. Chloroform extract was more efficient in inhibiting the bacteria than petroleum ether extract. However, such inhibition was obtained only at a huge concentration of 10mg/ml (Figure 1 and 2). *E.coli* exhibited strong resistance to both the extracts. The MIC values for *Salmonella Paratyphi A* petroleum ether extract was 0.56-0.63mg/ml and *Klebsiella pneumoniae* it was found to be between 5.8mg/ml to 6.1mg/ml in chloroform extract and in the case of petroleum ether extract, it was between 0.56-0.59mg/ml. Standard Andrographolide inhibited *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella Paratyphi A* effectively when compared to other bacterium, however no inhibition was recorded against *E.coli* (Figure 3). High performance liquid chromatography of the petroleum ether sample had a corresponding peak (Figure 4) as that of the standard and the peak was observed at 245nm with a retention time of 3minutes.

DISCUSSION

The anti-bacterial activity of Andrographolide proved that higher inhibition was obtained at a higher concentration of 100µg/ml in case of all 4 tested organisms. However, inhibition was also observed at a lower concentration of 10 and 50µg/ml. However, the absence of antibacterial activity of *Andrographis paniculata* extracts or Andrographolide on *Salmonella typhimurium*, *E.coli*, *Shigella sonnei*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* even at concentration of 100, 10 and 1mg/ml was reported earlier¹³. The present study revealed that the leaf extract was ineffective on *E.coli* alone. It is evident from the repeated work that aqueous extracts of *Andrographis paniculata* did not possess the active principle components which exhibited antibacterial activity. However the petroleum ether & chloroform extract showed an appreciable inhibitory activity. Minimum inhibitory concentration was determined & inferred that the minimum inhibitory concentration for *Salmonella Paratyphi A* in chloroform extract was between 5.8-6.0mg/ml and in petroleum ether extract was between 0.56-0.63mg/ml. Similarly, the minimum inhibitory concentration for *Klebsiella pneumoniae* was found to be between 5.8mg/ml to 6.1mg/ml in chloroform extract and in the case of petroleum ether extract, it was between 0.56-0.59mg/ml. It is evident that Andrographolide in the petroleum ether extract exhibited antibacterial effect on the test bacterium.

CONCLUSION

From the above results it is evident that the antibacterial activity of petroleum ether extract can be solely attributed to the presence of Andrographolide which seems to exhibit profound antibacterial activity on the gastrointestinal pathogens and is safe on *E.coli* at the tested concentration.

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