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Antimicrobial Potential Of Andrographis Paniculata Against Oral Pathogens

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Abstract: *Andrographis paniculata* (Burm. f.) Nees, a small medicinal plant, member of the family Acanthaceae, commonly known as Kalmegh (King of bitters) has been used in 26 Ayurvedic formulations as evidenced from Indian Pharmacopoeia. Ethanolic extract of *Andrographis paniculata* was studied for its antimicrobial activity against ten strains of oral *S.aureus*. It was observed that the extract was effective on all these oral *S. aureus*. Antibiotic resistance profile of oral *S.aureus* against four different antibiotics showed that 90% oral *S.aureus* were sensitive to Fluconazole and Streptomycin followed by 80% to Ampicillin and 70% to Vancomycin. *A. paniculata* extract was screened for five phytochemicals of which Saponins, Flavonoids, Terpenoids and Tannins were present.

Index Terms: *Andrographis paniculata,* Antimicrobial activity, Phytochemicals

I.INTRODUCTION

Medicinal plant is an integral part of human life to combat the sufferings from the dawn of civilization. The interest in medicinal plants has grown extremely from the use of herbal products as pharmaceuticals, cosmetics over the past twenty years. Plants are rich source of different types of medicines. Plants are also used to the scientific investigation of plants as self-medication by the general public for their biological effects in human beings. Most of the drugs used today are acquired from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine (Sukanya et al., 2009; Chaudhary et al., 2010). Antimicrobial compounds of medicinal plants have fewer side effects, less toxicity and they are also cost effective. The treatment with medicinal plants having antibacterial activity is potentially beneficial alternative and promising source of pharmaceutical agents (Sridevi et al., 2010). Therefore the use of plant extracts for antimicrobial activity against various diseases has been noticed to be helpful treatment since ancient time in Chinese medicine, Ayurveda, Arebic and Unani medicine (Tepe et al., 2004).

Andrographis paniculata is commonly known as green chiretta, Kalmegh. It is also known as "Bhui-neem," neem of ground and it is also called as 'King of Bitter'. It is an annual herbaceous, small, branched and erect plant in the family Acanthaceae. It is widely cultivated in Southern and South-eastern Asia and it is a native species of India, China, Java, Indonesia, Malaysia, Bangladesh, Sri Lanka and Pakistan. It is also cultivated in some parts of Europe. It is rich in wide variety of phytochemical constituents such as diterpenes, flavonoids and lactones (Chang, 1987). *Andrographis paniculata* is used in ample amount in Ayurveda, Unani and Siddha medicine as home remedy for many diseases not only in Indian traditional system but also in tribal medicine applications. *A. paniculata* is mostly used to get rid of body heat as well as it removes toxins from body (Dang, 1978). It is also used to prevent common cold and upper respiratory tract infections. *Andrographis paniculata* plant extract is used with known antimicrobial properties can be of considerable significances for therapeutic treatment. This plant also act as antidote against snake's and

insect's poisons (Samy et al., 2007). Thus, it is further reported that it shows the activities like hepatoprotective, antimalarial, antihypertensive, antipyretic, antithrombotic and antidote for snake bites. Anti-microbial compounds are of key interest nowadays in research areas due to the outbreak of newer infectious diseases, the increase in resistance of microorganisms towards the existing microbial compounds and harmful side effects of the compounds. For this reason, there is a need for the development of compounds with less side effects and more targeted activity on the microorganisms. Research is going on to identify compounds with anti-microbial activity mainly from plant origin as they have lesser side effects (Mishra and Tiwari, 2011). Oral diseases occur due to poor oral hygiene practices, leading to the accumulation of dental plaque on the teeth surface. Dental plaque is a complex biofilm made up of hundreds of oral pathogens (Benahmed et al., 2021). *Staphylococcus aureus* have been considered to be the most resistant species in the oral cavity and possible cause of failure of root canal treatment (Gomes et al., 2002). It is the part of microbiota with failed endodontic therapy and may be considered a dentinophilic microorganism (Siqueira and Rocas, 2009). The present investigation was undertaken to find out the antibacterial potentiality of the ethanol extract of *A. paniculata* against some oral pathogens.

II.MATERIALS AND METHODS

I. **Collection of *A. paniculata*-** The experimental plant species of *Andrographis paniculata* was collected from Shree Ram Nursery, Wardha (Maharashtra), India.

II. **Preparation of plant powder-** Fresh plants of *A. paniculata* were washed thoroughly in tap water followed by distilled water and were then shade dried. Dried plants were crushed and powdered using blender. Fine powder was obtained after sieving and stored in airtight container until further use.

III. **Preparation of plant extract-** The stored powder of *A. paniculata* was extracted with 70% ethanol. The successive extraction was done by a cold maceration process for seven days with regular agitation (Singh et al., 2007). After seven days of cold maceration process it was filtered through sterile muslin cloth and the solvent was evaporated using soxhlet apparatus. The residues obtained after evaporation were stored at -20ºC until used for experimentation (Geetha et al., 2017; Pandey et al., 2019).

IV. **Collection of Oral Pathogens-** To evaluate the antimicrobial activity of *A. paniculata* extracts, ten types of oral pathogens *Staphylococcus aureus* were collected from Pathology Laboratory in Nagpur.

V. **Antimicrobial test against oral pathogens-** Antimicrobial activity of plant extract of *A. paniculata* on oral pathogens was carried out by agar well diffusion method (Murry et al., 1995). Oral pathogens *S. aureus* strains were grown overnight on nutrient agar at 37^0C. The colonies were suspended in sterile saline water equivalent to a 0.5 MacFarland standard (1.5X108 CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread with the help of spreader. The wells of 6 mm diameter were cut into the agar medium with sterile cork borer. Then 20µl of plant extract was poured into wells with the help of micropipette. These plates were incubated for 24 hours at 37^0C. The diameter of the zone of inhibition around each well was measured and recorded (Bauer et al., 1966).

VI. **Antibiotic sensitivity test against oral pathogens-** Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer et al., 1966). Four different types of antibiotics were used in the study (Table 1). Oral pathogens *S. aureus* strains were grown on nutrient agar at 37^0C for 24 hours. The colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5X108CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread with the help of spreader. The antibiotic discs were placed on Hi-sensitivity agar. These plates were incubated for 24 hours at 37^0C. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant or sensitive" based on standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Table 1: Antibiotics used in the study

Sr. No.	Antibiotics	Abbreviation	Concentration
1.	Vancomycin	V	30 mcg
2.	Fluconazole	FLZ	25 mcg
3.	Streptomycin	S	100 mcg
4.	Ampicillin	A	10 mcg

VII. Phytochemical tests- Phytochemical analysis of *A. paniculata* extract was carried out to test saponins, glycosides, phytosterols, flavonoids, terpenoids, tannins (Pandey et al., 2019).

- **Test for Saponins:** 2g dried plant extract was boiled in 20ml distilled water and filtered. Then 10 ml of filtrate was mixed in 5 ml distilled water and vigorously shaken. 3 drops of olive oil was mixed in it and shaken vigorously again. Then the formation of emulsion observed for presence of saponins.
- **Test for Glycosides:** 5 ml HCl was added to plant extract and kept for 1 hour in water bath. Then 2 ml of Fehling's solution added (1 ml Fehling's A and 1 ml Fehling's B). 2 ml of extract was added then mixed properly and boiled. If there is appearance of yellow or red colour precipitate then it shows that there is presence of reducing sugar.
- **Test for Flavonoids:** Concentrated H_2SO_4 was added in extract. There was appearance of orange to crimson which shows the presence of flavonoides.
- **Test for Terpenoids:** 5g of plant extract was mixed with 2 ml of chloroform. 3 ml concentrated H_2SO_4 was added to it. A reddish brown coloured layer was formed which shows there is presence of terpenoids.
- **Test for Tannins:** 0.5g of plant extract was boiled in 20 ml of water in a test tube. After boiling it was then filtered. Then the few drops of 0.1% of ferric chloride were added. Brownish green colouration was observed which shows the positive result for tannins.

III.RESULTS AND DISCUSSION

In the present investigation, *A.paniculata* extract was tested against ten strains of *Staphylococcus aureus.* It was observed that the extract was effective on all these oral *S. aureus* (Table 2). In this study *A.paniculata* exhibited inhibitory activity against oral *S.aureus*. According to the results of Humnabadkar and Kareppa (2012); Hosamani et al., (2011), the *A. paniculata* showed maximum antibacterial activity against *Staphylococcus aureus.*Antibiotic resistance profile of oral *S.aureus* against four different antibiotics showed that 90% oral *S.aureus* were sensitive to Fluconazole and Streptomycin followed by 80% to Ampicillin and 70% to Vancomycin (Table 3).

Qualitative phytochemical analysis revealed that Saponins, Flavonoids, Terpenoids and Tannins were present in *A.paniculata* (Table 4) which is in concurrence of earlier reports of Polash et al., (2017); Sharma and Joshi, (2022). This present work was supported by the finding by Zhang et al. (2019) confirmed the remarkable antibacterial activity of bioactive compound; Andrographolide found in the *A.paniculata* plant. In direct bacteriostatic action, Andrographolide can prevent the formation of bacterial biofilms, the synthesis of virulence factors and bacterial adhesion. Thus all these phytochemicals act synergistically and exhibit beneficial effects in treatment of wide variety of disease.

IV.CONCLUSION

This study has proven the efficacy of the *A.paniculata* crude extract as a potential antimicrobial agent. Ethanolic extract of *Andrographis paniculata* was studied for its antimicrobial activity against ten strains of oral *S.aureus*. It was observed that the extract was effective on all these oral *S. aureus.* Antibiotic resistance profile of oral *S.aureus* against four different antibiotics showed that 90% oral *S.aureus* were sensitive to Fluconazole and Streptomycin followed by 80% to Ampicillin and 70% to Vancomycin. *A. paniculata* extract was screened for five phytochemicals of which Saponins, Flavonoids, Terpenoids and Tannins were present.

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Table 2: Antimicrobial activity *A. paniculata* extract against oral pathogens

Oral Pathogens	Zone of Inhibition
S. aureus 1	14 mm
S. aureus 2	17 mm
S. aureus 3	13 mm
S. aureus 4	22mm
S. aureus 5	17mm
S. aureus 6	18mm
S. aureus 7	20mm
S. aureus 8	14mm
S. aureus 9	13mm
S. aureus 10	19mm

Table 3: Antibiotic resistance profile of oral pathogens

Oral Pathogens	Antibiotics			
	Vancomycin	Fluconazole	Streptomycin	Ampicillin
S. aureus 1	12mm	17mm	23mm	21mm
S. aureus 2	Resistant	19mm	18mm	Resistant
S. aureus 3	14mm	16mm	21mm	13mm
S. aureus 4	19mm	22mm	Resistant	Resistant
S. aureus 5	16mm	20mm	21mm	20mm
S. aureus 6	13mm	24mm	25mm	22mm
S. aureus 7	17mm	19mm	22mm	24mm
S. aureus 8	Resistant	Resistant	17mm	20mm
S. aureus 9	19mm	19mm	18mm	18mm
S. aureus 10	Resistant	24mm	18mm	18mm

Table 4: Phytochemical analysis of *A. paniculata* plant extract

Phytochemical tests	Result
Flavonoids	+ve
Glycosides	-ve
Saponins	+ve
Tannins	+ve
Terpenoids	+ve

Where, +ve= Positive test, -ve= Negative test