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EVALUATION OF *IN - VITRO* ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF AQUEOUS STEM EXTRACT OF *CYNANCHUM ACIDUM (ROXB.) OKEN*

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ABSTRACT

The current research sought to evaluate the *in-vitro* antibacterial and antifungal activity of an aqueous stem extract of *Cynanchum acidum* (Roxb.) Oken. The disc diffusion and serial dilution methods were used to evaluate the antibacterial and antifungal activity of aqueous stem extract (100µl/ml). As a standard, the disc diffusion method was used with Ciprofloxacin (1mg/ml) for antibacterial activity and Fluconazole (1 mg/ml) for antifungal activity. When compared to the standard drug, the disc diffusion method revealed that the aqueous stem extract of *Cynanchum acidum* (Roxb.) is highly active against *B.subtilis* (G+ bacteria), *S.aureus* (G- bacteria), *P.aeruginosa* (G- bacteria), *A.niger* (Fungi) and *C.albicans* (Fungi). The serial dilution method was used to determine the minimum inhibitory concentration of aqueous stem extract against different micro-organisms. As a result, we believe that the aqueous stem extract of *Cynanchum acidum* (Roxb.) Oken may have therapeutic potential in the antibacterial and antifungal segments.

INTRODUCTION

1.1. Microbial infections

Infections caused by pathogenic microorganisms like viruses, fungus, and bacteria, have been a widespread issue for the population and are drastically on the rise. The propensity of microbes to cause disease in humans is a widespread issue that worries all areas of the health sciences. These microbes can cause a variety of diseases, and different therapy can be employed depending on the origin and forms of infection. [1] Acute lower respiratory tract infections, HIV/AIDS, gastrointestinal illnesses, TB, malaria, measles, tetanus, pertussis, sexually transmitted diseases (apart from HIV), and meningitis are some of them. [2]

1.2. Impact of bacterial infections

Bacterial antimicrobial resistance (AMR) is one of the public health challenges made worse by the ongoing pandemic and its exact scope is currently the subject of intensive research. According to the World Health Organization (WHO), 50% of all antibiotic regimens have been improperly given globally, with the alarming rise in AMR as the main effect. [3]

1.3. Impact of fungal infections

Globally and particularly in healthcare settings, the incidence rate of fungal infections has risen during the past few decades. The increased morbidity and mortality caused by fungi are caused by a variety of dangerous fungi, from mucosal candidiasis to nail or skin infections. Fungal disease is thought to affect more than more

than 80% of the world's population, or 5.7 billion people. The most common pathogen responsible for many instances of candidemia worldwide is *Candida albicans.* On the other hand, several non-*C. albicans* species, such as *C. parapsilosis, C. glabrata, C. tropicalis,* and *C.krusei,* have become important pathogens of fungal blood stream infections. [4]

1.4. Anti-microbial test methods

Testing for antimicrobial susceptibility can be used for drug development, epidemiology, and predicting treatment outcomes. Natural medicine has a tremendous impact on both the prevention and treatment of human disease. It has been demonstrated that a number of plant secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., have in vitro anti-bacterial properties.[5] A compound's antibacterial or antifungal activity may already be tested *in vitro* using several methods. Among these, dilution and diffusion techniques are the most often used. [6]

1.5. Plant used.

In this study we selected the stem of *Cynanchum acidum* (Roxb.) Oken plant **(Fig. 1)** *Cynanchum acidum* (Roxb.) Oken is a xerophytic plant belonging to the family of *Asclepiadaceae*. It belongs to the group of plants known as soma plants and is a traditional medicine used to make somras. It is a very branching, leafless shrub that clings to *Euphorbia caducifolia* Haines on China hills. [7] The numerous *C. acidum* plant parts, including the stem, root, seeds, latex, and fruits, demonstrated a variety of medicinal applications. This plant's juice is regarded as the divine beverage offered to the gods, considered to have therapeutic value and utilized as a natural health restorative that awakens and alerts the user.[8] Dog bites and otitis were treated with ear drops made from the plant's stem juice. But root was used to treat leprosy, rabies, emesis and snake bite. For cuts and open wounds, latex is used **(Fig. 2).** Several psycho-pharmacological effects, such as anti-psychotic, anxiolytic, and CNS inhibitory action, have been linked to the plant's entire extracts. [9]

Fig. 1: *Cynanchum acidum* (Roxb.) Oken plant

Fig 2: Latex of Cynanchum acidum[9]

1. MATERIALS AND METHODS

2.1. Preparation of plant extract

Plant materials were collected, dried in the shade, ground up using a mechanical mixer, and sieved using 40- and 60-mesh screens. Distilled water was used as the solvent during the decoction process to create the plant extract. It took 1000 ml of distilled water and 30 minutes to extract 50gm of the coarsely powdered stem of *Cynanchum acidum* (Roxb) Oken. Following that, Whattman no. 1 filter paper was used to filter the filtrate. In a water bath, the extract was further concentrated. The resulting extract was weighed and kept at 4°C for additional investigation. [10]

2.2. Phytochemical screening of aqueous stem extract of *C.acidum*

The presence of common phytoconstituent groups was detected in *C.acidum* aqueous stem extracts using modified methods. Saponins were detected using the froth test, alkaloids were detected by using Dragendroff's test, flavonoids were discovered using the alkaline reagent test. To

determine the presence of phenolic compounds and tannins, the ferric chloride test was used. The Keller-Killian test for cardiac glycosides and the Salkowski test for steroids and terpenoids were used to determine the presence of cardiac glycosides, steroids and terpenoids**.** [11]

2.3. *In vitro* anti-bacterial and anti-fungal activity by disc diffusion or agar diffusion method

Organisms used: Two G+ (*Bacillus subtilis, Staphylococcus aureus)* and two G- (*Pseudomonas aeruginosa, Escherichia coli)* bacteria strains and two fungal strains (*Aspergillus niger, Candida albicans*) were chosen for the current investigation.

Concentrations: Ciprofloxacin (antibacterial activity) and Fluconazole (antifungal activity) concentration is 10µg/disc and sample (aqueous stem extract) concentration is 100 µg/disc. [12]

Methodology: Bacterial and fungal inoculums (0.1ml) were spread on Nutrient agar (NA) and Sabouraud dextrose liquid (SDA) for 5 minutes before drying. For antibacterial and antifungal activity, Ciprofloxacin and Fluconazole were used as the positive controls, while 5% DMSO (Dimethyl sulfoxide) was used as the negative control. The bacterial plates ware incubated at 37^0C, while the fungal plates were incubated at 28^0C. The zone of inhibition (zone diameter) was measured after 24 hours for bacteria and 48 hours for fungal plates. Inhibition was detected when the zone diameter was greater than 6 mm.[13]**(Table: 1)**

2.4. *In vitro* anti-bacterial and anti-fungal activity by serial dilution method or turbidimetry method

Organisms used: Two G+ (*Bacillus subtilis, Staphylococcus aureus)* and two G- (*Pseudomonas aeruginosa, Escherichia coli)* bacteria strains and two fungal strains (*Aspergillus niger, Candida albicans*) were chosen for the current investigation.

Sample concentration: The concentration of aqueous stem extract is 1000 µl/ml. [14]

MIC tube concentrations: 8 MIC tubes concentration is 1000 µl, 500 µl, 250 µl, 62.5 µl, 31.25 µl, 15.63 µl for samples and 0 µl for control respectively.

Methodology: Minimum inhibitory concentration for bacteria and fungi were determined using Mueller Hinton broth (MHB) and Sabouraud dextrose liquid (SDL) medium. 1000 µl of MHB was transferred to MIC tubes of bacteria and 1000µl of SDL was transferred to MIC tubes of fungi. 1000µl of extract was pipetted into each first tube of bacteria and fungi. Serial dilutions were performed using a multichannel pipette so that each tube had 1000µl of the test material in serially descending concentrations. 10µl of bacteria and fungi cultures added to respective tubes. The bacterial tubes were incubated at 37^0C for 24 hours and the fungi tubes were incubated at 28^0C for 48 hours. Formation of turbidity was recorded as sign which indicates the microbial growth. The MIC value was determined as the lowest concentration at which no turbidity or visible of clear liquid occurs. [15] **(Table: 2)**

Table 1: Zone diameters of standard and sample

S.No	Micro organisms	Standard (10µg/disc)	Sample (100µg/disc)
1	*Bacillus subtilis* (G+)	40 mm	18 mm
2	*Staphylococcus aureus* (G+)	35 mm	17 mm
3	*Pseudomonas aeruginosa* (G-)	40 mm	18 mm
4	*Escherichia coli* (G-)	36 mm	10 mm
5	*Aspergillus niger* (Fungi)	26 mm	25 mm
6	*Candida albicans* (Fungi)	25 mm	18 mm

Table 2: Microbial growth and Minimum inhibitory concentration of sample and control

Micro organisms	Sample							Control
	1	2	3	4	5	6	7	8
	Concentration (µl)							
	1000	500	250	125	62.5	31.25	15.63	0
1. *Bacillus subtilis*(G+)	-	-	-	+	+	+	+	+
2. *Staphylococcus aureus* (G+)	-	-	-	+	+	+	+	+
3. *Pseudomonas aeruginosa*(G-)	-	-	-	-	+	+	+	+
4. *Escherichia coli* (G-)	-	-	-	+	+	+	+	+
5. *Aspergillus niger* (Fungi)	-	-	-	+	+	+	+	+
6. *Candida albicans* (Fungi)	-	-	-	-	-	+	+	+

(+) indicates the microbial growth

(-) indicates the Minimum inhibitory Concentration

Table 3: Minimum inhibitory concentration value of sample against different Micro organisms

S.No	Micro organisms	MIC (µl/ml)
1	*Bacillus subtilis* (G+)	250
2	*Staphylococcus aureus* (G+)	250
3	*Pseudomonas aeruginosa* (G-)	125
4	*Escherichia coli* (G-)	250
5	*Aspergillus niger* (Fungi)	250
6	*Candida albicans* (Fungi)	62.5

2. RESULTS AND DISCUSSION

The current study assessed the *Cynanchum acidum* (Roxb.) Oken aqueous stem extract's *in vitro* anti-bacterial and anti-fungal properties. Alkaloids, phenols, terpenoids, flavonoids, and other phytochemical elements have been confirmed by preliminary phytochemical testing of an aqueous stem extract.[11] The existence of these phytochemical constituents suggests that the plant stem may have anti-bacterial and anti-fungal action. Results from the disc diffusion method suggest that the aqueous stem extract of *Cynanchum acidum* (Roxb.) Oken has anti-bacterial and anti-fungal activity since the zone diameter of the sample (aqueous stem extract) is more than 6 mm. The serial dilution method was used to measure the aqueous stem extract's minimum inhibitory concentration **(Table 3)**.

CONCLUSION

According to the study, *Cynanchum acidum* (Roxb.) Oken's aqueous stem extract may have anti-bacterial and anti-fungal action; as a result, it may be utilized to treat bacterial and fungal illnesses.

According to the study, *Bacillus subtilis* (G+ bacteria), *Staphylococcus aureus* (G+ bacteria), *Pseudomonas aeruginosa* (G- bacteria), *Aspergillus niger* (fungi), and *Candida albicans* (Fungi) are all very susceptible to the *Cynanchum acidum* (Roxb.) Oken aqueous stem extract (Fungi).

To produce an appropriate formulation and determine the active principles causing anti-bacterial and anti-fungal action, more research is required.

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