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Antimicrobial Activity of *Alpinia calcarata* Rosc. and Characterization of New α, β Unsaturated Carbonyl Compound

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Abstract: The use of alternative medicinal therapy has increased the intrest of pharmacologists and herbalists over the past decade. Natural products from plants may offer new agents for antimicrobial use. A spectial feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity the so called secondary metabolites.Antibacterial of *Alpinia calcarata* Rosc. Shows significant results against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa*, *Enterobacter aerogenes*, *Salmonella paratyphi*, *Vibrio cholerae* and *Bacillus subtilis*. The methanol and hexane extract of *Alpinia calcarata* shared significant activity against selected bacterial strains.

Key words: Alpinia calcarata · Bacterial strains · Medicinal plants · Antimicrobial activity

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world [1]. The use of alternative medicinal therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug components, as plant derived medicines have made large contributions to human health and well being [2]. This is due to recognition of the value of traditional medicinal systems, particularly of Asian origin and the identification of medicinal plant from indigenous pharmacopeias, which have significant healing power [3]. Medicinal plants are distributed world wide, but they are most abundant in tropical countries [4-5]. Natural products from plant may offer new agents for antimicrobial use. A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity the so called secondary metabolites[5]. The wealth of medicinal plants and the tradictional knowledge drastically recede in the wake of burgeoning population pressure, acculturation, rapid modernization, multifarious human activities and various developmental activities [6].

Alpinia calcarata (Zingiberaceae) is a medicinl plant, used for several pharmaceutical purposes. The plants are widely used to relieving stomachache, treating colds, invigorating the circulatory system and reducing swellings [7]. *Alpinia calcarata* is cultivated in tropical countries including Sri Lanka, India and Malaysia. Leaves of the plant contain benzenoids such as protocatechnic acid, Vanillic acid and syringic acid, flavonoids and alkaloids [8].

Agar Disk Diffusion Method: The test compound (0.1 ml) was introduced into the disk and then allowed to dry. Thus the disk was completely saturated with the test compound. Then the disk was introduced on to the upper layer to the medium with the bacteria. The plants were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of the zone of inhibition. Chloromphenicol were used as the control.

The minimal inhibition concentration (mic) values were also studied for the microorganisms which were determined as sensitive to the extracts in the disc diffusion assay. A suspension of the tested microorganism

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MATERIALS AND METHODS

A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated in rotary shaker for 24 hr to activate the strain. Nutrient agar media (Himedia, Mumbai) were used for the study. The assay was performed using Agar disk diffusion method. The media were poured into Petri dishes. The test strain (0.2 ml) was inoculated into the media (inoculum size 10±8 cells/ml) when the temperature reached 40-42°C. care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions.

Microorganisms: The microbial strains are identified strains and were obtained from the National chemical Laboratory (NCL), Pune, India. Total eight Bacterial strains mostly recorded in hospital infection were used in this study namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa*, *Enterobacter aerogenes*, *Salmonella paratyphi*, *Vibrio cholerae* and *Bacillus subtilis*.

Preparation of Extracts: Dried plant material was suspended in 10 ml deionizer water and brewed as tea by boiling for 15 min. extracts were allowed to cool, centrifuged and the supernatants passed through 0.45µm and 0.22 µm filters, consecutively. Alternatively, 1 g dried plant material was extracted with 10 ml ethanol at room temperature for 20h, after which the extracts were filtered. For the antibacterial assay the ethanol extracts were dried and resuspended in distilled water.

RESULTS AND DISCUSSION

The antimicrobial activity of A. Calcarata extracts (Alcohol. Ethylacetate and Benzene) against the microorganisms examined in the present study and their potency, were qualitative assessed by the presence or absence of inhibition zones and zone diameter. The results are given in Table 1.

The results showed the alcohol extract of *A. calcarata* has an antibacterial effect against *K. pneumonia*, *Bacillus sabtillis* strains. This result confirms the previous studies which reported the methanolic extract of *Ocimum basillium* [9]. Ethylacetate and Benzene extracts showed the best inhibition zones for *Salmonella*, *Klebsialla* and *Bacillus* [10].

Most of the extracts tested showed some level of antibacterial activity. This supported the observations made by other investigators [11-13].

In addition, these results confirmed the evidence in previous studies reported that alcohol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, methanol and hexane [14-16].

In conclusion, the results obtained from our screening confirm the therapeutic potency of *Alpinia calcarata* analyzed and thus provide a rational for their use in traditional medicine. These results also form a good basis for selection of candidate plant species for further pharmacological and toxicity studies.

Hexane extract shows considerable activity against salmonella and Klebicilla studies on antimicrobial activity on *Gluzuma aulmifolia* on hexane extract also indicates the growth inhibition effect in selected bacterial strains [17]. Indian researches have shows antifungal and antihelmenthic activity [18] in extracts of *Alpinia calcarata*. However the plant is generally used in the Indian traditional medicine and it is recommended for as an abhrodisiac and a decoction is widely used in the treatment of bronchitis, cough, respiratory ailments, diabetics, asthma and arthritis [19]. The methanol and hexane extract of *Alpinia calcarata* shared significant activity against selected bacterial strains.

Table 1: effect of antimicrobial activity of Methanol, ethyl alcohol and hexane extract of *Alpinia Calcarata* on selected bacterial strains

S. No	Organism	Methanol extract	Ethyl alcohol extract	Hexane extract
1.	*Escherichia coli*	0.56±0.09	0.65±0.21	0.55±0.14
2.	*Staphylococcus aureus*	0.43±0.14	0.84±0.07	0.40±0.14
3.	*Pseudomonas aeroginosa*	0.44±0.01	0.68±0.14	1.31±0.45
4.	*Enterobacter aerogenes*	0.75±0.01	0.66±0.12	0.61±0.36
5.	*Salmonella paratyphi*	0.68±0.06	0.72±0.20	0.98±0.36
6.	*Streptococcus pneumoniae*	1.02±0.09	1.22±0.02	0.86±0.38
7.	*Bacillus subtilis.*	0.80±0.19	1.08±0.03	0.96±0.43
8.	*Vibrio cholerae*	0.73±0.16	0.83±0.12	0.63±0.18

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