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A STUDY OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE LEAVES OF *COLOCASIA ESCULENTA* LINN.

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Keywords:

Antibacterial, Antifungal, *Colocasia esculenta*, Zone of inhibition, Agar diffusion

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ABSTRACT: Aim: The study was done to evaluate the antibacterial and antifungal activity of the leaves of *Colocasia esculenta* Linn. **Materials and Methods**: Fresh tender leaves of *C.esculenta* collected, air-dried at room temperature, grounded to a fine powder and extracted with ethanol. The antibacterial and antifungal activity of *C.esculenta* compared to standard antibiotics was assessed by disc diffusion method by measuring the zone of inhibition. The organisms used for the test were *Staphylococcus aureus, Klebsiella, Escherichia coli* and *Pseudomonas aeruginosa* and *Candida albicans.* The agar disc diffusion method described by Kirby-Bauer was used for testing the antibacterial and antifungal activity. **Results**: Zone of inhibitions produced by sensitive organisms were demarcated by a circular area of clearing around plant extract impregnated discs and measured in millimeter (mm). Mean ± SEM was calculated and the data was statistically analysed by one way ANOVA followed by Dunnets multiple comparison test. **Conclusion**: In our study, ethanolic extract of *Colocasia esculenta* showed antibacterial activity against *S. aureus, P. aeruginosa, E.coli, Kleibsiella* and antifungal activity against *C. albicans* in a dose dependant manner.

INTRODUCTION: Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century [1]. Widespread and indiscriminate use of antibacterial agents resulted in development of drug resistance among many virulent pathogenic bacteria [2]. The clinical efficacy of many existing antibiotics is being threatened by rapid emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind [3]. *Colocasia esculenta* is herbaceous perennial plant belonging to the Araceae family [4]. They have a large corm on or just below the ground surface. The leaves are large to very large, 20–150 cm (7.9–59.1 in) long, with a sagittate shape. The "elephant's-ear" plant gets its name from the leaves, which are shaped like a large ear or shield. Both the roots and leaves are edible parts of the plant [5]. Originating in Asia, *C.esculenta* also known as 'Taro' is now primarily found in tropical and subtropical regions [6]. It is also known as *Arum esculentum* L. and *Colocasia antiquorum* Schott.[7]. It is commonly called as taro (English); alavi, patarveliya (Gujarati); arvi, kachalu (Hindi); alu (Marathi); alupam, alukam (Sanskrit); and sempu (Tamil), kosu (Assamese) [8, 9]. Fresh edible leaves of *Colocasia esculenta* form rich source of protein, ascorbic acid, dietary fibre [10] and some nutritionally important minerals and vitamins such as calcium, phosphorous, iron, Vitamin C, thiamine, riboflavin and niacin [6].

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In its raw form, the plant is toxic due to the presence of calcium oxalate and the presence of needle shaped raphides in the plant cells [5].

Scientific classification:

Kingdom: Plantae
Order: Alismatales
Family: Araceae
Subfamily: Aroideae
Tribe: Colocasiodeae
Genus: *Colocasia*
Species: *Colocasia esculenta* [5]

The herb has been known since ancient times for its curative properties and has been utilized for treatment of various ailments such as asthma, arthritis, diarrhea, internal hemorrhage, neurological disorders, and skin disorders [6]. Whole plant is crushed into paste and applied on insect stings, cuts, burns and internal hemorrhage [11]. The study was done to evaluate the antibacterial and antifungal activity of the leaves of *Colocasia esculenta* Linn.

MATERIALS AND METHODS:

Collection, Identification and Extraction of Plant:

Materials: Approximately 1 kg of fresh tender leaves of *C.esculenta* collected during July-August 2014 was used for the study. The plant material was air-dried at room temperature. The dried leaves were grounded to a fine powder and stored in an air tight container.

Preparation of the Extract: Two hundred and fifty grams of the dry powder obtained was subjected to continuous Soxhlet extraction. The extract was collected in Petri dishes [12]. The extract was concentrated in vacuum using a rotary flash evaporator. There was a net yield of 22.6 g of the concentrated extract (9.12%). The extract was stored in a refrigerator at 4°C in labeled air-tight containers for further use. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antibacterial activity.

Drugs and chemicals used in the study:

a) Ethanolic extract of leaves of *C.esculenta* (EECE)
b) Antibiotic discs obtained from Ranbaxy Pharmaceuticals.
c) Petridises
d) Agar obtained from Himedia Laboratories, Mumbai.

The present study was conducted in the Department of Pharmacology and Department of Microbiology, Gauhati Medical College, Guwahati after getting approval from Institutional Ethics Committee (No.MC/233/2013/264).

Evaluation of Antibacterial and Antifungal Activity:

Test organisms: The organisms used for the test were *Staphylococcus aureus* (*S. Aureus), Klebsiella sp, Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Candida albicans* (*C.albicans*). The stock cultures were obtained from Department of Microbiology, Gauhati Medical College, Guwahati, Assam, India.

Preparation of plant extract impregnated discs: Whatman filter paper no.1 was used to prepare discs of 5mm. Sterilization was done by autoclaving and subsequently dried at 80ºC for an hour in hot air oven. Then the discs were impregnated with ethanolic extracts of *C.esculenta*. Each produced disc have the ability to absorb about 0.01ml.

Testing method for Antibacterial and Antifungal activity: The modified agar disc diffusion method originally described in 1966 by Kirby-Bauer [13] was used for testing the antibacterial and antifungal activity. Overnight culture of bacterial and fungal strains was done in nutrient agar (HiMedia, Mumbai) at 37±2°C. A loopful of isolated colony of the overnight grown culture was inoculated in 4ml of Peptone water (HiMedia, Mumbai) at 37°C for 2 h. The turbidity of resulting suspension was compared to 0.5 McFarland turbidity standards. The level of turbidity was equivalent to approximately 3.0×10^5 cfu/ml. The Mueller Hinton Agar media (HiMedia, Mumbai) was prepared and poured into Petri dishes. Once the media solidifies it was then inoculated with microorganism suspended in peptone water.

The filter paper discs were impregnated with the extracts and placed individually on the Mueller-Hinton agar with flamed forceps and gently pressed down to ensure contact with the agar surface.

The discs were placed far enough from each other to avoid both reflection waves and overlapping rings of inhibition. DMSO was used as negative control. The experiment was performed at four different concentrations (50, 100, 200 and 400mg/ml). The Petri dishes were incubated for 24 h at 37±2°C for the bacteria. Sabouraud agar was used for *C.albicans* and tested by the same method as described above. The Zone of inhibitions produced by sensitive organisms were demarcated by a circular area of clearing around *C.esculenta* plant extract impregnated discs and were compared with zone of inhibitions of standards (Gentamicin 10µg, Ceftazidime 30µg and Fluconazole 25µg) and control (DMSO). The tests were repeated five times to ensure reliability.

Statistical Analysis: Statistical Analysis of the data was done by using One-way ANOVA with Dunnett's posthoc test using Graph pad prism software. *P* value <0.05 was considered statistically significant.

RESULTS: In our study, ethanolic extract of *C. esculenta* showed antibacterial activity against *S. aureus, P. aeruginosa, E.coli, Kleibsiella* and antifungal activity against *C. albicans* in a dose dependant manner.

The results were found to be statistically significant when compared with the standard drugs Gentamicin and Ceftazidime ($p<0.05$) for antibacterial activity and the standard drug Fluconazole ($p<0.05$) for antifungal activity. However, the antibacterial and antifungal activity at dose EECE (100mg/ml) does not show significant difference when compared to EECE (200mg/ml). Also, there is no significant difference between dose EECE (200 mg/ml) and EECE (400mg/ml).

TABLE 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *COLOCASIA ESCULENTA* (EECE) BY MEASURING THE ZONE OF INHIBITION IN AGAR DISC DIFFUSION METHOD

	Measurement of Zone of inhibition (in mm)				
	Staphylococcus aureus	***Pseudomonas auruginosa***	***Escherichia coli***	***Kleibsiella***	***Candida albicans***
DMSO (Control)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EECE (50mg/ml)	3.3 ± 0.32 ^{a,b}	4.2 ± 1.1^{a,b}	4.6± 0.67^{a,b}	3.5 ± 0.76^{a,b}	5.7 ± 2.1^{a,b}
EECE(100 mg/ml)	8.24± 0.66 ^{a,b,c}	9.32 ± 0.43^{a,b,c}	9.21±1.4^{a,b,c}	8.21± 1.5^{a,b,c}	6.3±0.78^{a,b,c}
EECE(200 mg/ml)	11 ± 1.21^{a,b,d}	14.6± 1.54^{a,b,d}	16.2±1.2^{a,b,d}	13.8±0.76^{a,b,d}	11.54±1.2^{a,b,d}
EECE(400 mg/ml)	14.3 ± 1.45^{a,b}	17. 4 ± 0.57^{a,b}	17.1±0.62^{a,b}	16.4± 0.43^{a,b}	16.2 ± 0.54^{a,b}
Gentamicin (10 µg)	25.06 ± 0.25^{a}	26.12 ± 0.34^{a}	28.04 ± 1.1^{a}	28.32± 0.43^{a}	…….
Ceftazidime (30 µg)	25.67 ± 0.78^{a}	25.07 ± 1.2^{a}	25.65±0.65^{a}	24.23± 0.12^{a}	……..
Fluconazole (25 µg)	…….	……	…….	…….	28.42±0.65^{a}

Values are expressed as Mean ± SEM (n=5); One Way ANOVA followed by Dunnett's multiple comparison tests.
a- $p<0.05$ when compared to control, b- $p<0.05$ when compared to standard, c- $p>0.05$ when compared to EECE(200 mg/dl), d- $p>0.05$ when compared to EECE (400mg/dl)

DISCUSSION: In our study, it is seen that ethanolic extract of the leaves of *Colocasia esculenta* shows antibacterial activity against *S. aureus, P.aeruginosa, E.coli* and *Kleibsiella* and antifungal activity against *C.albicans*. Govindrajan *et al* showed various solvent extracts of leaves exhibited inhibitory property against *S. aureus, Staph. Epidermis, Bacillus cereus, Streptococcus fecalis* [14]. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population [15]. The zone of inhibition with EECE (400mg/ml) was 17. 4 ± 0.57 for *P.aeruginosa* which correlate with the result obtained in another study Fahmeeda *et al* who studied the antibacterial activity of *Aegle marmelos* [16]. In a similar study Dhanraj BN. *et al* , it was seen that various extracts of the leaves of *Colocasia esculenta* exhibited antibacterial activities against *P. aeruginosa, E. coli, Bacillus, Salmonella typhi* etc [17]. Yang et al. evaluated the antifungal activity of *C.esculenta,* along with molecular cloning and recombinant gene expression studies [18].

Phytochemical studies demonstrated the presence of beta-carotene, iron, folic acid alkaloids, alkyl-resorcinols, glycosides, phenolics, saponins, sterols, essential oils, resins, numerous sugars and organic acids in *Colocasia esculenta* [19].

Secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides etc, which have been found *in vitro* to have antimicrobial properties [20, 21]. An antifungal compound, 9, 12, 13-(trihydroxy-(E)-10-octadecenoic acid, and two enzymes, lipoxygenase and lipid hydroperoxide-converting enzyme, which are responsible for the production of antifungal lipid peroxides, were detected in taro tubers infected by *C.eratocystis* fimbriata [4]. Medicinal plants possessing such phyto constituents bear high potential for inclusion into the world of antimicrobial drugs.

CONCLUSION: Considering the results obtained in the study, we can assume that the ethanolic leaf extract of *Colocasia esculenta* Linn. might become a useful component in the treatment of bacterial and fungal diseases. The development of more purified products and the active components responsible for its antibacterial and antifungal property of *Colocasia esculenta* should be emphasized as well as the inclusion of the herbal medicines in the treatment of various infections should be encouraged. Further well designed studies are necessary to throw light o the various uses of herbal drugs for the benefit of mankind.

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