

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ETHANOL EXTRACT OF *CURCUMA CASIEA*

Curcuma casiea (Black Turmeric) is a kind of turmeric with bluish-black rhizome, famous for its unique medicinal properties. It is a perennial herb, belonging to Zingiberaceae (Ginger) family, native to North-East and Central India. The leaves have a deep violet-red patch which runs through the length of the lamina. Usually, the upper side of the leaf is rough and velvety, but this character may vary. Flowering bracts are green with a rustic tinge. Flower petals may be deep pink or red in color. The inside of the rhizome is deep-blue or bluish-black in color. The rhizome is bitter, hot taste with pungent smell. The name 'black turmeric' holds due to the presence of cells related to black color in the rhizome. The rhizome slightly resembles the light-grey rhizome of *Curcuma aeruginosa*. Scientific researches on the volatile rhizomes oil of *Curcuma casiea* resulted in the identification of 30 components, representing 97.48% of the oil, with camphor (28.3%), ar-turmerone (12.3%), (Z)-ocimene (8.2%), ar-curcumene (6.8%), 1, 8-cineole (5.3%), -elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and -curcumene (2.82%) as the major constituents. *Curcuma casiea* was claimed to be useful in treating piles, leprosy, bronchitis, asthma, cancer, epilepsy, fever, wounds, impotency, fertility, menstrual disorders, tooth ache, vomiting etc.

Curcuma casiea is still widely used as a medicinal plant around the world. Microorganisms have developed resistance to many antibiotics and this has created immense problem in the treatment of infectious diseases. This situation forced scientists to search new antimicrobials. Secondary metabolites proved to be the most important group of compounds that showed wide range of antifungal and antibacterial activity (Ahmed *et al.* 2002, Aureli 1992, Rahman *et al.* 1999). So, there are continuing needs for new antibacterial and antifungal agents since none of the available drugs are free from adverse effects and limitation. Considering the value of plants and other natural products as agents for infectious disease treatment, an attempt was made by present study to evaluate the effect of ethanol extract of *Curcuma casiea* as antimicrobial agent.

Curcuma casiea rhizomes were collected from Shilak dak Rangamati, Chittagong, Bangladesh. Rhizomes were cut into small pieces (1-2cm), air dried, powdered mechanically and extracted with 90% ethanol. The extract was filtered & concentrated to near dryness under reduced pressure and low temperature (40-50⁰ C) with the help of the rotary vacuum evaporator. A measured amount of the test sample was dissolved in definite volume of ethanol to give solution of known concentration (2 mg/ml).

The acute toxicity test aims at establishing the therapeutic index, i.e. the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The greater the index, the safer is the compound. Because of species variation, several species of animals are used to determine the LD50 or median lethal dose. In our study, the LD50 of the extract of *Curcuma casiea* is 80 mg/kg [I.V.in](#) mice. The doses of 10 mg/kg, 20 mg/kg & 30 mg/kg were used to see the antineoplastic activity of *Curcuma caesia*.

To find out the effect of *Curcuma casiea* extract (whole rizome) on the morality of brine shrimp noupilii median lethal concentration of brine shrimp (LC50) was measured and it was found to be 7.20 µg/ml.

Bacterial test organisms were *Bacillus cereus* BTTC 19, *Bacillus subtilis* BTCC 17, *B. megaterium* BTCC18, *Shigella dysenteries* AE 14396, *Shigella sonnei* ICDDR'B *Salmonella typhi* AE 14612, *Staphylococcus aureus* ATTC 6538, *Escherichia coli*. ATCC 25922, *Vibrio cholerae* (INABA-ET) AE 14748, *Pseudomonas sp.* The bacterial strains were collected from the Institution of Nutrition and Food Science, Dhaka University and ICDDR'B, Dhaka. Fungal test organisms were *Aternaria alternata* (Fr.) Keissler, *Botryodiplodia theobromae* Pat. *Colletotichum corchori* Ikata (Yoshida), *Curvularia lunata* (Wakker) Boedjin, *Fusarium equiseti* (Corda) Sac and *Macrophomina phaseolina* (Maulbi) Ashby. Fungal strains were collected from Dept. of Microbiology, Chittagong University.

The *in vitro* antibacterial activity of the extract was done following the agar cup plate method (Rao & Nigam 1970) and nutrient agar was used as basal medium for bacterial growth. Hundred ml of the extract (2 mg/ ml) was poured into the agar cup. The plates were kept in the freeze for diffusion and then incubated at 37⁰C for 24 to 48 hours. The activity was expressed in terms of the

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *C. CASIEA*

diameter of zone of inhibition in mm. All the results were compared with standard antibiotic Ampicillin (20 µg/ disc).

The *in vitro* antifungal activity of the extract was determined following the poisoned food technique of Grover and Moore (1962) using potato dextrose agar medium. The extract was dissolved in ethanol & then mixed with sterilized PDA to obtain a final concentration of 2 mg/ml. Nystatin was used as standard antifungal antibiotic (100 µg/ml) for comparison of results under identical conditions. The inoculated plates were incubated at $25^{\circ} \pm 1^{\circ} \text{C}$ for 3-5 days. The percentage inhibition of radial mycelial growth of test fungi was calculated as follows :

$$I = \frac{(C-T)}{C} \times 100$$

Where, I =Percent of inhibition,

C = Diameter of fungal colony in the control,

T = Diameter of fungal colony in the treated.

The antibacterial activity of alcoholic extract of *Curcuma casiea* was determined and the result is presented in the table-1. It was found that the degree of susceptibility varied depending on the species. The extract showed broad spectrum antibacterial activity against all the test organisms except *Shigella dysenteriae* and *Shigella sonnei*.

TABLE 1: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *CURCUMA CASIEA*.

Zone of inhibition in of <i>Curcuma casiea</i> in mm.										
Bacterial test organisms										
Name of the plant extract	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Bacillus megateium</i>	<i>Shigella sonnei</i>	<i>Shigella dysentriae</i>	<i>Salmonella typhi</i>	<i>Vibrio cholera</i>	<i>Escherichia coli</i>	<i>Pseudomonas sp.</i>	<i>Staphylococcus aureus</i>
<i>Curcuma casiea</i>	17	20	17	-	-	21	27	23	12	27
* Ampicillin	25	22	22	20	35	30	30	16	13	-

(-) Indicates no zone of inhibition

* Standard antibiotic

However the highest inhibition was recorded with *Vibrio cholera* and *Staphylococcus aureus* (27mm). Table –2 represents the antifungal activity of alcoholic extract of *C. casiea*. The rhizome extract of *C. casiea* showed wide range of antifungal activity with marked radial mycelial growth inhibition against all the phytopathogens tested. However the highest antifungal activity was recorded against *Macrophomina phaseolina* (77.44%).

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *C. CASIEA*

TABLE 2: ANTIFUNGAL ACTIVITY OF ETHANOL EXTRACT OF *CURCUMA CASIEA*.

Percent radial mycelial growth inhibition						
Name of the plant extract	<i>Alternaria alternata</i>	<i>Curvularia lunata</i>	<i>Fusarium equiseti</i>	<i>Macrophomina phaseolina</i>	<i>Botryodiplodia theobromae</i>	<i>Colletotrichum corchori</i>
<i>Curcuma caseia</i>	60.20	67.18	50.26	72.44	56.52	64.30
*Nystatin	55.55	72.41	45.79	70.78	82.35	42.00

*Standard antibiotic

Medicinal plants are an important source of new compound for the discovery and development of chemotherapeutic agents. So *C. casiea* used in traditional and folk medicine may serve as a potential source of natural antibacterial and antifungal agent.

REFERENCES

AHMED, A.M.A., RAHMAN, M.S., AND ANWAR, M.N. 1999. Antimicrobial activity of extracts and crude alkaloids of *Polyalthia longifolia* (Sonn.) *Chittagong Univ. J. Sciences.* **23** (1):53-56

AURELI, P., COSTANTINI, A. AND ZOLEA, S. 1992. Antimicrobial activity of some plant essential oil against *Listeria monocytogenes*. *J. Food Science.* **55**:344-348

- GOVER R.K AND MOORE J.D.1962. Toximetric studies of fungicides against brown rot organisms *Sclerotinia fluticola* and *S. laxa*. *Phytopathol.* **52**:876-880
- RAHMAN, M.S., BEGUM, J., CHOWDHURY, J.U. AND ANWAR, M.N.1998. Antimicrobial activity of *Holarrhena antidysenterica* stem bark. *Bangladesh J.Microbiol.***16**(2):101-105
- RAO, N.B.V. AND NIGAM, S.S. 1970. The in vitro antimicrobial efficiency of essential oils. *Indian J. Med. Res.* **58**: 627-633.
-

ZULKARNAIN CHOWDHURY, MOHAMMAD SAYEDUL ISLAM, MOHAMMAD RAZUANUL HOQUE, A.M.MASUDUL AZAD CHOWDHURY¹, ROBIUL HASAN BHUIYAN AND MOHAMMAD ALAUDDIN*.

Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong 4331, Bangladesh.

¹Department of Genetic Engineering and Biotechnology, University of Chittagong, Chittagong 4331, Bangladesh.

* Corresponding author

Manuscript received on 24. 5. 2010; Accepted on 29.9.11
The Chittagong University Journal of Biological Sciences, Vol. 5 (1 & 2). Page No:149-154