

ANNEXURE-III  
DEPARTMENT OF SCIENCE, TECHNOLOGY, ENVIRONMENT

Proforma for furnishing Research Project proposals to DSTE.

1. TITLE OF THE PROJECT : THE ANTI MICROBIAL ACTIVITY OF LATEX AND AERIAL PARTS PLANT EXTRACT OF CALOTROPIS GIGANTEA (L.) R.BR.
2. NAME OF THE PRINCIPAL INVESTIGATOR AND ADDRESS FOR COMMUNICATION WITH PHONE NUMBER/E-MAIL.  
  
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3. DESIGNATION AND NAME OF THE INSTITUTION IN WHICH WORKING.  
LECTURER IN ZOOLOGY, DEPARTMENT OF ZOOLOGY, TAGORE ARTS COLLEGE,  
LAWSPET, PUDUCHERRY-605008.
4. NAME OF THE CO-INVESTIGATOR (IF ANY) & ADDRESS FOR COMMUNICATION WITH PHONE NUMBER/E-MAIL
5. OBJECTIVES OF THE PROJECT (NOT MORE THAN 10 LINES)  
RESIDUAL PESTICIDES IN SOIL OR ON CROPS ARE TRANSPORTED FROM FIELDS TO SURFACE AND GROUND WATERS. THE LEAVES AND STEMS OF *CALOTROPIS GIGANTEA* (L.) R.BR. ARE USED IN AGRICULTURE FOR SOIL FERTILITY & INSECTICIDAL ACTIVITY. IF THEY ARE RELEASED IN ENVIRONMENT IN ADEQUATE BEFORE DEGRADATION, THEY KILL NON-TARGET AND OTHER AQUATIC SPECIES, SOME TOXIC SUBSTANCES BIO-ACCUMULATE IN TISSUES OF FISH AND OTHER SPECIES THUS POSING HEALTH RISKS TO HUMAN BEINGS AND OTHERS, WHO CONSUME THE FISH AND CONTAMINATED WATER. DUE TO WHICH THE NUTRITIVE VALUE OF FISHES GETS REDUCED; FISH POPULATION GRADUALLY DIMINISHES BECAUSE OF THE TOXICANTS. IT IS BEEN KNOWN IN MEDICINAL IMPORTANCE IN SHRIMPS (SEMBV VIRAL INFECTION) AND PREVENTS VIRAL WHITE SPOT DISEASE (RAMAN, 1997).
6. LIKELY BENEFITS OF THE PROJECT.  
TOXIC POLLUTANTS RENDERED ALMOST LIFELESS BY A LETHAL COMBINATION OF AGRICULTURAL FERTILIZERS, PESTICIDES, INDUSTRIAL POLLUTANTS, SEWAGE RUNOFF. THE PROCESS KILLS THE FOOD CHAIN FROM THE BOTTOM UP, RENDERING THE AREA VIRTUALLY LIFELESS. THE INTERACTION AMONG VARIOUS CHEMICAL POLLUTANTS IN THE AQUATIC SYSTEM MAY BE SYNERGISTIC, ANTAGONISTIC OR ADDITIVE, AND MAY CAUSE ACUTE TOXIC EFFECT ON DIFFERENT SPECIES. BIOPESTICIDES OF ANY TYPE HAVE BEEN APPROVED FOR USE BY PRODUCERS IN THE DEVELOPING WORLD WHERE, CHEMICAL USE IS LARGELY OR TOTALLY UNCONTROLLED. THE BIOACTIVE CHEMICAL SUBSTANCES ARE FOUND IN PLANTS SPECIES THAT SHOW DELETERIOUS EFFECTS ON FISHES AND OTHER NON-TARGET ORGANISMS LEADING TO DEATH. VERY LESS DATA IS AVAILABLE ON THE BIOPESTICIDAL EFFECT ON FISHES. THE HAZARDS OF BIOPESTICIDES USAGE HAVE AWAKENED THE MODERN MAN TO REALIZE THE RISK INVOLVED IN CONSTANT USE OF PESTICIDES.

HENCE, AN ATTEMPT IS MADE TO INVESTIGATE THE EFFECTS OF LATEX, PLANT EXTRACT AND THE RECOVERY OF LATEX TOXICITY WITH THE ADDITIVE NUTRIENTS. LATEX OF *C.GIGANTEA* (L.) R.BR. AND THE EXTRACTS OF AERIAL PARTS OF THE PLANT ARE USED AS ARROW POISON, INFANTICIDE, SLOWS ANTI IMPLANTATION ACTIVITY, HOMICIDAL POISON AND FERTILIZER AND ALSO EFFECTIVE FISH POISON, INSECTICIDAL, GOOD OVICIDAL AND LARVICIDAL IN NATURE.



IT DESCRIBES THE STUDIES ON THE IMPACT OF LATEX AND PLANT EXTRACT OF *CALOTROPIS GIGANTEA*(L.)R.BR. ON FRESHWATER FISH, *ANABAS TESTUDINEUS*,(BLOCH) A COMMONLY EDIBLE FISH AND WIDELY CULTIVATED IN INDIA. THE IMPACT WAS ASSESSED IN TERMS OF MEDIAN LETHAL CONCENTRATION OF LATEX AND PLANT EXTRACT OF *CALOTROPIS GIGANTEA*, (L.)R.BR. THE IMPACT WAS ASSESSED IN TERMS OF BEHAVIOURAL, NUMBER OF BIOCHEMICAL, HAEMATOLOGICAL PARAMETERS AND HISTOPATHOLOGICAL CHANGES IN THE FISH EXPOSED TO THE SUBLETHAL CONCENTRATIONS OF LATEX, PLANT EXTRACT AND THE RECOVERY OF LATEX TOXICITY WITH THE ADDITIVE NUTRIENTS STUDIED.

#### ACTIVE PRINCIPLES

*CALOTROPIS* SPECIES CONTAINS THE PHYSIOLOGICALLY ACTIVE COMPONENTS NAMELY -

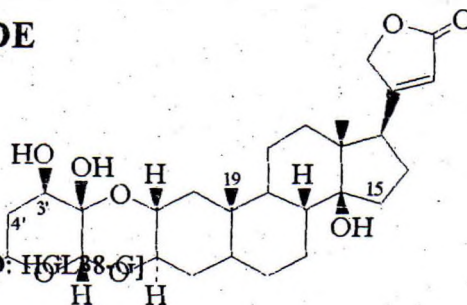
CARDIAC GLYCOSIDES (CARDENOLIDES) - CALOTROPIN, USCHARIN, VORUSCHARIN (DIHYDROUSACHARIN), CALOTOXIN, CALACTIN, USCHARIDIN, GIGANTIN, TRITERPENES, PENTACYCLIC TRITERPENOID AND FLAVONOID TRIGLYCOSIDES (VONBRUSCHWEILER ET AL.,1969;MARTIN ET AL.,1979) .THESE COMPOUNDS ARE HIGHLY TOXIC (KIUCHI ET AL.,1998;HAVAGIRAY ET AL,2004).

THEY HAVE DIRECT EFFECT ON HEART AND CENTRAL NERVOUS SYSTEM. CALOTROPAGENIN IS THE COMMON AGLYCONE OF ALL THE GLYCOSIDES CALOTROPIN, GIGANTIN AND USCHARIN SHOW DIGITALIS-LIKE ACTION ON THE HEART. MANY MEMBERS OF ASCLEPIADACEA FAMILY ARE TOXIC AND

GLYCOSIDES ARE OFTEN THE CULPRIT. CAUSE GASTRO-INTESTINAL TOXICOSIS. INHIBIT CELLULAR MEMBRANE ATPASE ENZYME SYSTEM ACTIVITY.

DURING EARLY COURSE OF POISONING, ANIMALS EXHIBIT RAPID BREATHING .CONVULSIONS, IRREGULAR HEART ACTIVITY. CATTLE AND HORSES CONSUMING CARDIAC GLYCOSIDE-CONTAINING PLANTS ARE OFTEN FOUND DEAD. POSTMORTEM FINDINGS INCLUDE HEMORRHAGES. CONGESTION, EDEMA AND CELL DEGENERATION OF THE ORGANS INCLUDING MULTIFOCAL MYOCARDIAL DEGENERATION AND NECROSIS (KNIGHT AND WALTER, 2002).

#### GOMPHOSIDE



CHAPMAN&HALL NO: HGL 28-61

CAS NO: 36597-51-0

COMPOUND CODE: VTO750XA2760 MOLECULAR FORMULA:

$C_{29}H_{42}O_8$

MOLECULAR WEIGHT: 518.287

BIOLOGICAL SOURCE: CARDIAC GLYCOSIDE

PHYSICAL DESCRIPTION: CRYSTALLINE(MeOH)

MELTING POINT: 268-271

**GAMPHOSIDE DERIVATIVES  
CALACTIN**

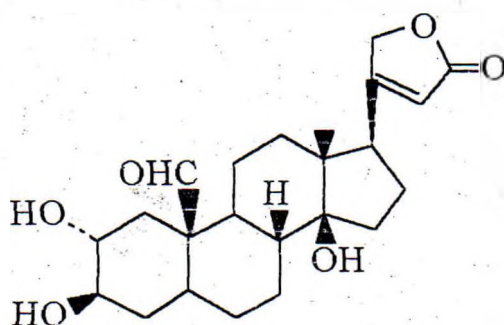
CHAPMAN&HALL NO: BZK24  
CAS NO: 20304-47-6  
COMPOUND CODE: AJO750VTO750XA2760  
MOLECULAR FORMULA: C<sub>29</sub>H<sub>40</sub>O<sub>9</sub>  
MOLECULAR WEIGHT: 532.63  
PHYSICAL DESCRIPTION: CRYSTALLINE (MeOH)  
MELTING POINT: 270-272

**GOMPHOTOXIN (or) CALOTROPIN**

CHAPMAN&HALL NO: HDG2-E  
CAS NO: 1986-70-5  
COMPOUND CODE: AJO750VTO750AJ5000  
MOLECULAR FORMULA: C<sub>29</sub>H<sub>40</sub>O<sub>9</sub>  
MOLECULAR WEIGHT: 532.63  
USE: AFRICAN ARROW POISON  
PHYSICAL DESCRIPTION: CRYSTALLINE  
TOXICITY(CAT): 0.1mg/Kg

**CALOTOXIN**

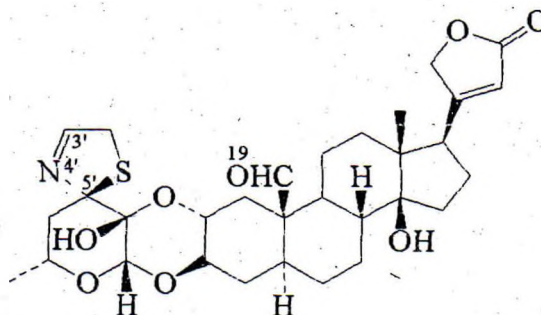
CHAPMAN&HALL NO: HDD88-M  
CAS NO: 20304-49-8  
COMPOUND CODE: AJO750VTO750  
MOLECULAR FORMULA: C<sub>29</sub>H<sub>40</sub>O<sub>10</sub>  
MOLECULAR WEIGHT: 548.629  
PHYSICAL DESCRIPTION: CRYSTALLINE (MeOH)  
MELTING POINT: 270-272



CHAPMAN&HALL NO: HGP48-D  
CAS NO: 24211-64-1  
COMPOU CODE: AJO750UTO750  
MOLECULAR FRMULA: C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>  
MOLECULAR WEIGHT: 404.502  
BIOLOGICAL SOURCE: AGLYCON  
PHYSICAL DESCRIPTION:  
CRYSTALLINE  
MELTING POINT: 248-252  
(Sing., 1972; Lardon, 1969; Lardon, 1970).

**USCHARIN**

CHAPMAN&HALL NO: HGK16-T  
CAS NO: 24211-81-2  
COMPOUND CODE: AF7700AJO750VX6790VE7700VTO750  
MOLECULAR FORMULA: C<sub>31</sub>H<sub>41</sub>NO<sub>8</sub>S  
MOLECULAR WEIGHT: 587.733  
BIOLOGICAL SOURCE: LATEX-ALKAOID  
MELTING POINT: 270-271  
TOXICITY: CARDIAC POISON



**VORUSCHARIN (dihydrouscharin)**  
(DERIVATIVE OF USCHARIN)

Neem	<i>Azadirachta Indica</i>	Meliaceae	Insecticidal, repellent, antifeedant	All plant parts
Persian Lilac	Melia azedarach	Meliaceae	Contact and stomach poison, insecticidal, repellent,	Dried leaves
Pyrethrum	<i>Chrysanthemum Cineraria</i>	Compositae	Pure contact poison. Insecticidal,	Flowers
Quassia	<i>Quassia amara</i>	Simarubaceae	Contact and stomach poison . Insecticidal,	Stem & leaves
Ryania	<i>Ryania</i>	Flacourti	Contact and	Dried roots, leaves
Sabadilla	<i>Schoenocaulon officinale</i>	Lilaceae	Contact and stomach poison.	Seeds
Sweet flag	<i>Acorus calamus</i>	Araceae	Insecticidal repellent,	Dried rhizomes
Turmer	<i>Curcuma</i>	Zingiber	Insecticidal and	Root
Andean lupin	<i>Lupinus mutabilis</i>	Lamiaceae	Antifeedant Nematicidal fungicidal	Seeds

## 7. METHODOLOGY

### CALOTROPIS GIGANTEA

BELONGS TO FAMILY: ASCLEPIADACEA

DISTRIBUTED IN TROPICAL & SUB-TROPICAL ASIA AND AFRICA EXTENDING INTO ARABIA, WEST INDIES & TROPICAL SOUTH AND CENTRAL AMERICA. FOUND IN ALL PLAINS OF

BLACK COTTON SOIL IN WASTE PLACES & ON ROAD SIDES.

TALL LATICIFEROUS SHRUBS OR SMALL TREES, COMMONLY KNOWN AS THE SWALLOW WORT OR MILKWEED - 2 TO 3M HIGH

FLOWERS INODOROUS, PURPLISH OR WHITE IN UMBELLATE LATERAL CYMES.

THEY ARE GOOD SOIL-BINDERS, AND ARE RECOMMENDED FOR DESERTS, HAVE A LIFE SPAN OF 12 YEARS. SEEDS NUMEROUS. VIDARBHA AND IN MANY PARTS OF SOUTH INDIA, THEY FLOWER AND FRUIT THROUGHOUT THE YEAR (Nadkarni, 1991 ; The Wealth of India, 1992).



THE PLANT EXTRACT SHOWS CYTOTOXIC ACTIVITY (SMITH ET AL.,1995)

ANTI PLASMODIAL AND LARVICIDAL (SHARMA AND SHARMA,2001;MOURSU,1997)

PROLONGED HIGH DOSES CAUSE HEADACHE, BURNING IN MICTURITION AND LEUCORRHOEA,

WIDE SPREAD TESTICULAR NECROSIS AND DAMAGE TO LIVER WHEN ADMINISTERED ORALLY TO DESERT GERBIL.

THE DRUG IS HIGHLY TOXIC.

HIGHER DOSES CAUSE VOMITING DIARRHEA, BRADYCARDIA AND CONVULSIONS

THESE MEDICINAL PLANTS PRODUCE TOXIC EFFECTS ON THE ANIMAL SYSTEM, IF NOT USED CAREFULLY OR IN REGULATED AMOUNT. (KHARE, 2004).

OTHER USES:

C.GIGANTEA (L.)R.BR. LEAVES ARE USED AS GREEN MANURE FOR BETEL NUT, PADDY AND WHEAT;

REPORTED TO CORRECT ALKALINITY IN SOIL. COMPOST CAN ALSO BE MADE OUT OF IT.

THE STEM BARK YIELDS RESINS AND WAX (THE WEALTH OF INDIA, 1992). LEAVES AND FLOWERS ARE WIDELY USED ALL OVER INDIA FOR WORSHIP IN TEMPLES.

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## **WATER QUALITY ANALYSIS**

THE WATER QUALITY WAS DETERMINED PERIODICALLY ACCORDING TO STANDARD METHOD (APHA, 1998).

**TEMPERATURE, DISSOLVED OXYGEN, pH, CO<sub>2</sub>, HARDNESS(CACO<sub>3</sub>), TOTAL ALKALINITY.**

### **3. BEHAVIORAL STUDIES**

BEHAVIORAL STUDIES WERE CARRIED OUT AGAINST LATEX TOXICITY AND ALSO BIOPESTICIDE MADE OUT OF CALOTROPIS GIGANTEA. THE NUMBER OF TIMES EACH TEST INDIVIDUALS **SURFACED** WAS OBSERVED FOR A KNOWN PERIOD (5-10MIN.) AT 9.30 A.M., 11.30 A.M. AND 3.30 P.M. THE **DISTANCE TRAVELED** BY THE INDIVIDUALS PER DAY WAS ESTIMATED BY MULTIPLYING THE MEAN NUMBER OF VISITS. NUMBER OF **OPERCULAR MOVEMENTS** WAS OBSERVED FOR 1 MINUTE USING A STOP WATCH, AND THE MEAN VALUES WERE TAKEN TO GET THE BEHAVIORAL ACTION OF THE PARTICULAR FISH. THE **OPTOMOTOR RESPONSE** OF THE CONTROL AND EXPERIMENTAL FISH WERE MEASURED USING THE **OPTOMOTOR RESPONSE**

### **4. HAEMATOLOGICAL STUDIES**

THE PRESENT STUDY WAS UNDERTAKEN TO TRACE THE EFFECTS OF SUB-LETHAL CONCENTRATIONS OF LATEX, LATEX WITH SUPPLEMENTS AND PLANT EXTRACT OF *CALOTROPIS GIGANTEA*(L.)R.BR. ON BLOOD OF *ANABAS TESTUDINEUS*.(BLOCH). THE PARAMETERS STUDIED INCLUDES **HEMOGLOBIN CONTENT (HB) -SAHLIS METHOD**, **OXYGEN COMBINING CAPACITY(DECIE AND LEWIS, 1963)**, **TOTAL RED BLOOD CORPUSCLES COUNT (RBCHAEMATOCRAIT (HT) (SNIESZKO, 1960).** **MEAN CORPUSCULAR HEMOGLOBIN (MCH) (DECIE AND LEWIS, 1963),** **,MEAN CORPUSCULAR VOLUME (MCV) (DECIE AND LEWIS, 1963),** **, MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) (DECIE AND LEWIS, 1963), AND** **TOTAL WHITE BLOOD CELL COUNT,** TO EXPLAIN HOW THE TEST COMPOUNDS PRODUCES CHANGES IN ERYTHROPOIETIC ACTIVITY.