



## BIOLOGICAL STUDIES OF THE SEED OF *IPOMOEA MURICATA* JACQ; LINNE (CONVOLVULACEAE)

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Ethnomedical information from old books authored by Dominican friars stated that seeds of *Ipomoea muricata* also known as "Pepitas del maravillosas" or "Pepitas del Tonkin" were traditionally used for different types of skin ailments such as ulcers, wounds, lesions, boils, burns, insect and snake bites, etc. The ground seeds were also taken orally for gastric and duodenal ulcers. It was also said to possess analgesic and anti-inflammatory properties.

To prove scientifically its claimed medicinal value, a research programme on the chemical and biological characterization of the biologically active constituents of the plants was undertaken by the University of Santo Tomas Research Centre for the Natural Sciences.

The purpose of the study was to :

- establish the 7-day median lethal dose (LD<sub>50</sub>) ;
- prove its antimicrobial activities ;
- determine the basic pharmacological effects on animal models ;
- establish the claimed analgesic property ;
- determine the mutagenic/or antimutagenic effects ;
- determine the anti-inflammatory effects and
- determine any adverse effects of the biologically active constituents of the plant

### Botanical studies

The *Ipomoea muricata* plant of the "Tonkin" plant is an annual vine growing several meters high on trellises for support. Stems are herbaceous and rough due to the presence of many minute spiculate protuberances growing from the epidermis. The leaves are simple, smooth, soft and entire, the base cordate, the lobes rounded and the apex tapers into a short caudex. The light green palmately netted-veined blades are 4-9 cm wide and 6-10 cm long, while the petioles reach up to 10 cm in length. The inflorescence is axillary, one to few flowered. Each flower is perfect and is held by a peduncle somewhat shorter than the petiole. The corolla which opens at night is actinomorphic, pink-purple, campanulate and funnel-shaped. The fruit is a thin walled capsule, dehiscent by valves, ovoid with a diameter of 0.8-1.5 cm. Two to four glabrous, beige colored seeds are formed in each capsule.

The scientific identification of the plant was confirmed by local botanists and Dr Van Oostroom of Rijksherbarium of Leiden, Netherlands.

### Microbiological assays

The crude hydroalcoholic extract of the seeds which was obtained by single solvent extraction using 80% ethyl alcohol and subsequently evaporated into a syrupy consistency by the use of a rotavap was subjected to *in vitro* microbiological assays. Paper disc, agar cup and the turbidimetric methods and standard ATCC Gm(+), Gm(-), *Mycobacterium* 607 and fungal organisms were used for the tests.

The microbiological assays using the paper disc method showed that the extract is active against Gm(+) standard test microorganisms such as *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633 and *Mycobacterium* 607 but has no activity against Gm(-) standard test microorganisms e.g. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27852 and also on fungal organisms e.g. *C. albicans* and *S. cerevisiae*.



**Microbiological assay of 10% and 30% hydroalcoholic extract of *I. muricata* seeds.**

% Conc.	Length of storage	Zone of Inhibition in mm					
		Sa	Ps	Ec	Bs	Ca	Sc
10%	1-7 days	20	-	-	21	-	-
	4 weeks	20	-	-	22	-	-
	8 weeks	18	-	-	20	-	-
30%	1-7 days	18	-	-	21	-	-
	4 weeks	20	-	-	21	-	-
	8 weeks	19	-	-	20	-	-
Solvent control		-	-	-	-	-	-

Legend :  
 Sa - *Staphylococcus aureus* ATCC 24923  
 Ps - *Pseudomonas aeruginosa* ATCC 27852  
 Ec - *Escherichia coli* ATCC 25922  
 Bs - *Bacillus subtilis* ATCC 6633  
 Ca - *Candida albicans*  
 Sc - *Saccharomyces cerevisiae*

A comparative study of the alcoholic extracts of the seed harvested in 1961, 1967 and 1980 was conducted to evaluate the effect of storage on the antimicrobial activity. Stability tests indicated diminishing activity with storage periods for the test organisms *S. aureus* and *B. subtilis*; however effect on *Mycobacterium* 607 was found to be unaffected by storage.

Stability to heat were also determined. The antimicrobial activity of the seed extract was found to be thermostable even after heating the extract for one (1) hour at 120°C.

Turbidimetric method was used for the determination of the minimum bactericidal concentration. The alcoholic extract of the seeds was found to have a minimum bactericidal effect against *S. aureus* at a concentration of 500 µg/ml; at a concentration of 300 µg/ml, the extract showed only a bacteriostatic effect.

Microbiological assays of the clinical isolates were also performed. The Gm(+) clinical isolates obtained from wound infections, which ranged from wound abscesses to wound secretions, of different volunteer subjects responded well to the extract whereas Gm(-) clinical isolates did not respond in conformity with results obtained using the standard tests organisms.

**Preliminary microbiological assay of the alcoholic extract of *I. muricata* seeds against clinical isolates.**

Isolate N°	Clinical Isolates		Zone of inhibition in mm
	Gm (+)	Gm (-)	
1	<i>Staphylococcus aureus</i>		42
2			40
3			40
4		<i>Escherichia coli</i>	11
5			-
6		<i>Pseudomonas aeruginosa</i>	10

**Pharmacological-toxicological determinations**

The pharmacological-toxicological, analgesic and antimutagenic effects and anti-inflammatory property of the crude alcoholic extract of the plant were established using experimental Swiss mice and guinea pigs. The standard acute toxicity tests to determine the LD<sub>50</sub>



and any adverse effects, modified acetic acid and toepinching method for analgesic activity determinations, micronucleus test to determine the mutagenic and/or antimutagenic effects, the cotton pellet granuloma formation method and the rat paw edema method using the plethysmometer to determine the anti-inflammatory property were performed.

Pharmacological-toxicological determinations showed that the crude extract has an LD<sub>50</sub> of 1100 mg/kg body weight (IP) and 7810 mg/kg body weight (oral). Slight depression manifested as decrease in motor activity, loss of screen grip and righting reflex, ataxia, analgesia, diarrhea and loss of appetite were observed using the multidimensional screening method. Hematological examinations did not reveal significant effects on the blood cellular elements.

**The calculated 7-day LD<sub>50</sub> of the crude alcoholic extract of the seed of *Ipomoea muricata*.**

Routes of Administration	Litchfield & Wilcoxon Method
Intraperitoneal injection	1100 mg/kg body weight (493-2453 mg/kg bw)
Oral	7810 mg/kg body weight (1963-7963 mg/kg bw)

The calculated LD<sub>50</sub> values indicated that the crude alcoholic extract of *Ipomoea muricata* seeds is slightly toxic (based on the T. Loomis category of toxicity) when administered intraperitoneally and orally.

Determination of the analgesic property using the modified Acetic Acid and Toepinching Method and experimental Swiss mice indicated that the effect of the crude extract is comparable to the analgesic effect of 112mg/kg body weight of acetylsalicylic acid.

Micronucleus test revealed that 50 mg, 100 mg and 250 mg/kg body weight of the crude extract was anti-mutagenic. However, at very high doses, the non-nitrogenous ether soluble fraction was found to be mutagenic.

The plant extract showed a slight anti-inflammatory activity.

#### Pharmaceutical studies

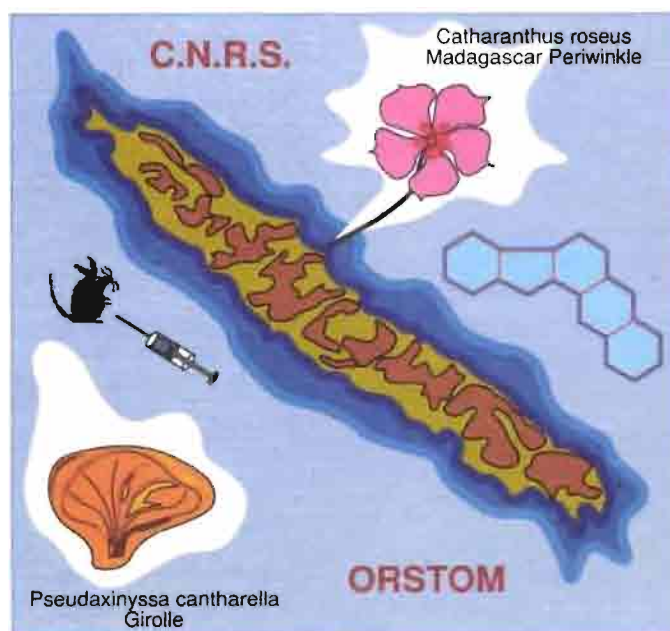
The UST RCNS has developed different pharmaceutical dosage forms of the alcoholic extract of the seeds of the plant - an ointment preparation for pyogenic skin lesions, tincture as an antiseptic, throat swab preparation for throat infections and an otic preparation for externa otitis media.

These different preparations have undergone double blind clinical trials on human volunteers of the UST Charity Hospital. Results indicated that they are efficacious and safe to use.

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## ACTES



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