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Investigation of Cytotoxic Potential of Ethanolic Extract of *Citrus limetta* fruit peel, *Paederia foetida* Leaves and Methanolic Extract of *Cuscuta reflexa*.

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In this study, the comparative cytotoxic potential of *Citrus limetta*, *Paederia foetida* leaves *Cuscuta reflexa* plant were investigated. The Brine shrimp lethality bioassay was used to evaluate cytotoxicity. These extracts showed remarkable cytotoxic activity, LC50 value of these extracts were 74.814 µg/ml, 42.57µg/ml, 24.013µg/ml respectively compared to vincristine sulphate, a reference drug, LC50 0.52 µg/ml. These leaves extracts of have excellent cytotoxic potential and comparative study is demonstrated here with figures and tables. Among all three extracts *Cuscuta reflexa* has more cytotoxic potential than both.

Keyword: *Citrus limetta*, *Paederia foetida*, *Cuscuta reflexa*, Cytotoxic Potential, Vincristine Sulphate, LC50, EECL, EEPF, MECR.

1. Introduction:

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. The definition of Medicinal Plant has been formulated by WHO (World Health Organization) as follows- "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." *Citrus limetta* is a species of citrus. Common names for varieties of this species include sweet limetta, Mediterranean sweet lemon, sweet lemon, and sweet lime. It is a small tree which may reach 8 m in height. The sweet lemon has irregular branches, and relatively smooth, brownish-grey bark. It possesses numerous thorns which may grow to

anywhere from 1.5 to 7.5 cm long. In Iran it is called Limu Shirin. In India, it is commonly called as Moosambi (Kannada), sathukudi (in Tamil), mousambi, mosambi, or musambi (in Hindi/Urdu). In Bangladesh it is known as malta. *Paederia foetida* is a species of plant, with common names that are variations of **Skunkvine**; **Stinkvine**; or **Chinese Fever Vine**. It is native to temperate and tropical Asia; and has become naturalized in the Mascarenes, Melanesia, Polynesia, and the Hawaiian Islands. *P. foetida* is native to Bangladesh and southern Bhutan; Cambodia; Taiwan and China (in Hong Kong and Macau, and the provinces of Anhui, Fujian, Gansu, Guangdong, Guangxi, Hainan, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Shanxi, Sichuan, Xizang, Yunnan,

Zhejiang); India (in Andhra Pradesh, Warangal, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, in the northern part of West Bengal, and the Andaman and Nicobar islands); Indonesia; Japan (in Honshu, Kyushu, Shikoku prefectures, as well as in the Ryukyu Islands); Laos; Malaysia; Myanmar; Nepal; the Philippines; Singapore; South Korea; Thailand; and Vietnam. This is an interesting parasitic plant named as Dodder in English. In our country Bangladesh, we call this as Swarna Lota, Alok lota, Swarna lotika etc. Botanical name of this plant is *Cuscuta reflexa*. A yellow, leafless, fleshy, twining parasite, branches stout, fleshy, forming dense yellow masses on shrubs and low trees; racemes lax, 2.5-10.0 cm long, clustered or sub-spicate; flowers sub-cylindric, 0.8 cm long, scales prominent; capsules tough, fleshy, sub-quadrate; seeds large, black.

2. Plant Material:

The plant *Citrus limetta* was collected from the local market of Chittagong City, and *Paederia foetida* and *Cuscuta reflexa* were collected from Chittagong Hill Tracts and all samples were authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh. The plants *Citrus limetta*'s fruit peels and *Paederia foetida*'s leaves were collected, dried and extracted with ethanol and the whole plant of *Cuscuta reflexa* was dried and extracted with methanol. Then the ethanolic extract of *Citrus limetta* (EECL) and *Paederia foetida* (EPPF) and methanolic extract of *Cuscuta reflexa* (MECR) were used for brine shrimp lethality bioassay to investigate cytotoxic potential.

3. Cytotoxicity Test:

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [2], [3]. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to

mature shrimp called nauplii. The brine shrimp lethality bioassay was performed to predict the cytotoxic activity [2], [6] of the *Citrus limetta*, *Paederia foetida* Leaves, *Cuscuta reflexa*. For experiment, The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations 90.91 µg/ml, 74.07 µg/ml, 56.60 µg/ml, 38.46 µg/ml, 19.60 µg/ml and 9.09 µg/ml, 7.40 µg/ml, 5.60 µg/ml, 3.85 µg/ml, 1.97 µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulphate was used as positive control. [4], [5] then matured shrimps were applied to each of all experimental vials and control vial. After 24 hrs, the vials were inspected using a magnifying glass and the number of survived naupili in each vials was counted. The mortality end point of this bioassay was defined as the absence of control forward motion during 30s observation. [7] From this data the percent of lethality of the brine shrimp naupili for each concentration and control was calculated. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality [8] was plotted on the graph paper and the values of LC₅₀ were calculated Using Microsoft excel 2007.

4. Result and Discussion:

All extracts showed remarkable cytotoxic activity. LC₅₀ value of EECL, EPPF and MECR were 73.55µg/ml, 51.59µg/ml, 35.75µg/ml respectively compared to vincristine sulphate, a reference drug, LC₅₀ 0.52 µg /ml. All three extracts have more or less cytotoxic potential which is demonstrated here with figures and tables [Table 1 and Figure 1 and 2]. Among all three extracts, the MECR has more cytotoxic potential.

Table 1: Cytotoxic activity of EECL, EEPF and MECR

Concentration (C); $\mu\text{g/ml}$	LogC	No. of nauplii taken	No. of nauplii dead in EECL	No. of nauplii dead in EEPF	No. of nauplii dead in MECR	% Mortality of EECL	% Mortality of EEPF	% Mortality of MECR
6.25	0.7959	20	1	2	2	5	10	10
12.5	1.0969	20	2	3	4	10	15	20
25	1.3979	20	6	5	9	30	25	45
50	1.699	20	8	8	11	40	40	55
100	2	20	10	15	16	50	75	80
200	2.301	20	14	17	19	70	85	95
400	2.6021	20	18	19	20	90	95	100

Figure 1: Determination of LC_{50} value for EECL, EEPF and MECR from linear correlation between log concentrations versus % Mortality.

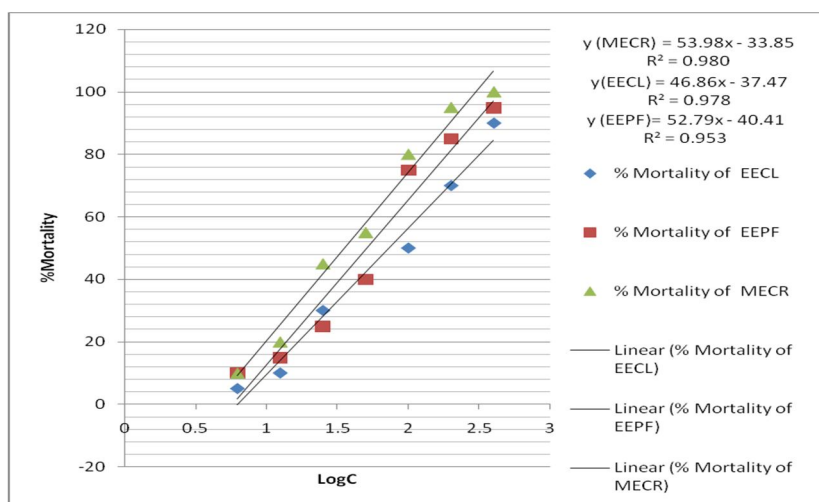
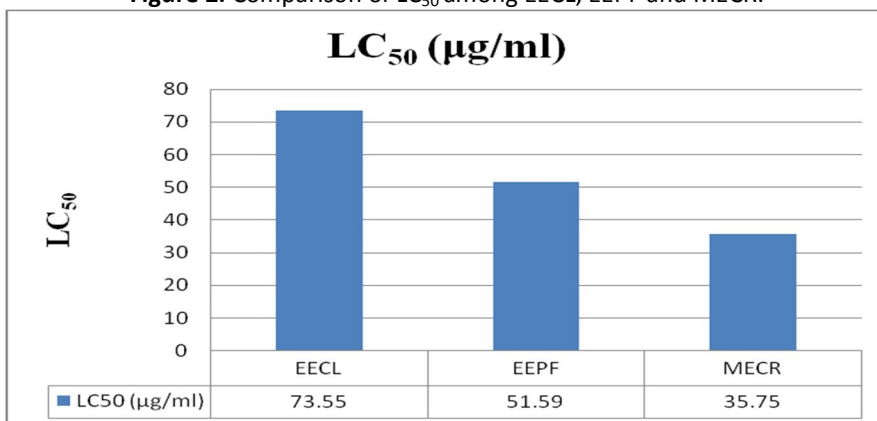


Figure 2: Comparison of LC_{50} among EECL, EEPF and MECR.



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