direct extraction of cells, or callus culture in
lts. To be able to perform the extraction of
necessary first cell suspension cultures. Cell
n cultures, can be obtained by growing
orm callus or crumbs from leaf mesophyll cells
able to obtain cell suspension cultures from
phyll of which required several stages of cell
from leaf mesophyll tissue, purification cells
ulture. At the time of cell isolation, there are
ctors that affect success of cell isolation.
ors, among others, the source of explant, type
ntration of enzyme pectinase, osmoticum, long
time and method of incubation and pH
nzyme.
t this stage of cell suspension cultures, often
variety of media both on the composition of
dia, culture and explant own environment to
secondary metabolite production [4]. The
on of sugar in the medium range growth of 1-
-10% for secondary metabolite production
The goal of this research was to determine the
and the concentration of leaf *C.asiatica*
e R-10 which can generate a number of cells
viability for leaf mesophyll cell suspension
asiatica. The second aim is to investigate the
different concentrations of sucrose on the
medium on the growth of cell suspension
the formation of compounds asiaticoside.

CH METHODS

ls Research

of explant

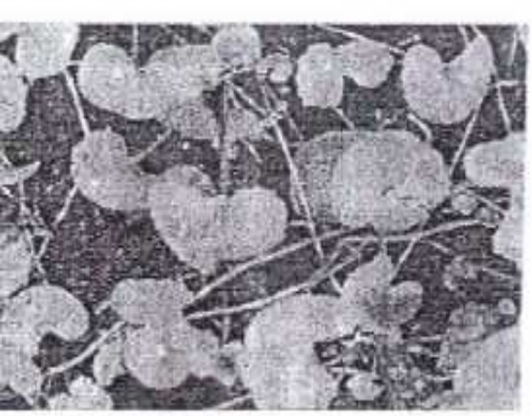

Fig.1. *Centella asiatica* L.Urban

ion chemicals

oap, bayclin, 96% alcohol, sterile distilled

on medium

rvinylpyrrolidone from Sigma, sorbitól,
2-N-Morpholino ethane sulphonic acid
macerozyme R-10 from Yakult Co. HONSA., I
Japan.

4. Purification medium

KNO3, Ca (NO3) 2, sorbitol and MES

5. Cells suspension culture medium

For cell suspension culture used Murashige and
medium or MS medium, plant growth regulator
(Indole 3-Acetic Acid) and BAP (Benzylaminopur

6. Fenosafranin 0.2% solution of the sigma f
observation of cell viability

C. Research Tools

1. **sterilization** ; cell isolation and purification o
and cell culture were as follows: laminar air flo
lamp equipped, autoclave, incubation space, ligh
disecting set, magnetic stirrer, analytical scales,
equipment, centrifuges and conic tube , paper s
0.22 µ milipore from Whatman, nylon mesh size
the light microscope, photomicroskop, pH paper,
umbrellas, micrometers, hemocytometer, cou
aluminum foil, shakers, oven, pipette, holder
incubators.

2. **chemical analysis**

Glass tools for the extraction of cells, tools for
layer chromatography, UV lamp 254 nm and 26
Shimadzu brand, bath water, and eksikator.

Procedure

At this stage of isolation and purification of meso
cells, the experimental design used was compl
randomized design factorial. The first factor is
sequence of leaf: leaf sequence-1 (D1), 2nd (D2) a
(D3). Macerozyme second factor is the concentratio
R-10 is E0 = 0% (0g), E1 = 0 , 1% (1g / l), E2 =
(2g / l) and E3 = 0.3% (3g / l). Each combinatio
treatments with three replicates. The param
measured were the number of cells and cell viab
The data obtained were analyzed by ANOVA
Duncan Test 's Multiple Range Test (DMRT) at
confidence level. While at this stage of cell suspen
cultures tested the addition of sucrose treatment on
production medium with a concentration of 0%, 2.
5% and 7.5%. To measure the growth of both
suspension cultures in growth medium and the med
of production is done by measuring packed cell volu
(PCV) in a certain time interval.

RESULT AND DISCUSSION

1. Obtaining the number and viability of cells