# Evaluation of Antimicrobial Activity of *In Vivo* and *In Vitro* Produced Saponins of *Convolvulus Pluricaulis* Choicsy

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## ABSTRACT

The development of resistant microorganisms on prolonged exposure to existing antimicrobial agents has been known for a long time. This has led to the continual search for ways of eradicating resistant strains of micro organisms. *Convolvulus pluricaulis* Choicsy a well known medicinal plant of Convolvulaceae family is used in India for hundreds of years. Tissue culture protocol was developed for *in vitro* production of saponins through leaf culture on MS media with 1 mg/l 2, 4-D and 1 mg/l kinetin. Analysis of *in vivo* and *in vitro* produced saponins of *Convolvulus pluricaulis* through HPTLC revealed 13 fluorescent zones in UV-200 nm and 11 fluorescent zones in UV-254 nm. The antimicrobial activity of *in vivo* and *in vitro* produced saponins investigated against *Escherichia coli* (gram negative), *Staphylococcus aureus* and *Bacillus subtilis* (gram positive) bacteria revealed that activity of *in vivo* produced saponin was especially higher against *Staphylococcus aureus* followed by *Bacillus subtilis* and *Escherichia coli* on the basis of inhibition zone area but in case of *in vitro* produced saponins *Escherichia coli* was most sensitive in comparison to other two microorganisms.

**Keywords**: *Convolvulus pluricaulis,* Saponins, Antimicrobial activity

## INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant material (WHO, 1993). The scientific studies available on a good number of medicinal plants indicates that promising phytochemicals can be developed for many human health problems (Gupta, 1994), including diabetes, cancer and infectious diseases. The continued investigation into the secondary plant metabolites for antiinfective agents

has gained importance because of the alarming increase in the rate of resistance of pathogenic microorganism to existing antibiotics. Therefore the need to develop efficient, safe and inexpensive drugs from plant sources is of great importance.

Plant tissue culture technology offers an alternative to fulfill the demand of raw material with continues supply and also in conservation of the endangered medicinal plants (Erdei et al. 1981, Shoyama et al. 1983, Huang et al. 2000). Callus culture is an effective tool to obtain the pharmaceutically important compounds in *in-vitro* condition which enables the plants to be preserved in their natural habitat.

*Convolvulus pluricaulis* (L.) Choicy (Shankhpushpi) of family Convolvulaceae is an important and well known traditional source of medhya rasayan drug (that counteract stress and improve intelligence and memory) used for rejuvenation of brain and mental health and to promote intellect and memory (Dixit, 1971; Kapoor, 1990). It is also used as a psycho-stimulant and tranquilizer. It is reported to reduce mental tension (Barar and Sharma, 1965 and Mudgal, 1972). Saponins are important active substances of this plant known for eml v lidi cidem. In view of the growing demand of the plant, their short supply and threats of losing them in future tissue culture studies were conducted. Saponins were extracted form *in vivo* leaf and *in vitro* callus produced materials, quantified and were tested for its antimicrobial activity on the bacterium strain *Escherichia coli*, *Bacillus subtilis* and *Staphylococus aureus*.

## MATERIALS AND METHODS

Plants of *Convolvulus pluricaulis* were collected from the Gujarat University Botanical Garden. Collected material was thoroughly washed with water followed by double distilled water. Leaves were surface sterilized with 0.1% (w/v) systemic fungicide - bavistin followed by 0.1 % (w/v) $HgCl_2$, 5% (v/v) sodium hypochlorite (NaOCl) and 5% (v/v) Tween-20 solution respectively and finally rinsed for 4-5 times with sterile double distilled water and used for culture on MS media described by Murashige and Skoog (1962) medium supplemented with 2,4-D and kinetin in combination of 1:1, 2:2, 3:3, 4:4 and 5:5 mg/l and 4% (w/v) sucrose and 0.8% (w/v) agar-agar. The cultures were maintained in culture room at 25?1? C and exposure of 12 hr. to fluorescent light. The callus was harvested at the end of 8 weeks. Saponins were extracted from the leaves and the callus using standard method of Daniel, 1991.

For Antimicrobial activities of *in vivo* and *in vitro* produced saponins were performed using the agar diffusion method of Boakye and Yiadom, 1979. *E. coli, S. aureus and B. subtilis* was inoculated on nutrient agar plate and spread uniformly using a glass spreader. These test organisms were obtained from Microbiology Department of Gujarat University. With the help of sterilized pipette, 0.8 ml extracts was introduced into the well bored onto the surface of the culture. Control experiments with methanol, solvent ether and ethyl acetate were set up against *in vivo* and *in vitro* produced saponins. The plates were allowed to stand for one hour at room temperature to allow the diffusion of the substances to proceed before the growth of organism commenced. The plates were finally incubated at $37^0$C for 24hrs.

## RESULT AND DISCUSSION

Of the various combination tried MS media supplemented with 1 mg/l 2,4-D and 1 mg/l kinetin proved to be the best for callus growth from the leaf of *C. pluricaulis.* Study of *in vivo* and *in vitro*

produced saponins of *C. pluricaulis* through HPTLC in UV-200 nm revealed 13 fluorescent zones. Seven spots resolved at Rf 0.01, 0.12, 0.24, 0.32, 0.33, 0.47 and 0.64 were produced common in both the sample *in vivo* and *in vitro*. Substance resolved at Rf 0.05, 0.20, 0.64 and 0.91 were restricted to *in vivo* sample only while two distinct spots at Rf 0.22 and Rf 0.80 were noticed only from *in vitro* sample. 11 fluorescent zones of saponins produced *in vivo* and *in vitro* were reported from chromatogram studied in UV-254 nm. Four saponins produced from both the sample were resolved at Rf 0.17, 0.24, 0.64 and 0.91. Two saponins synthesized only in *in vivo* sample were resolved at Rf 0.04 and Rf 0.20. Five new substances from *in vitro* samples were resolved at Rf 0.13, 0.26, 0.35, 0.51 and 0.76. The antimicrobial activity of *in vivo* and *in vitro* produced saponins of *C. pluricaulis* was investigated against *Escherichia coli* (gram negative), *Staphylococcus aureus* and *Bacillus subtilis* (gram positive) bacteria. Activity of *in vivo* produced saponin was especially higher against *S. aureus* followed by *B. subtilis* and *E. coli* on the bases of inhibition zone area but in case of *in vitro* produced saponins *E. coli* was most sensitive in comparison to other two microorganisms. It concludes that *in vivo* produced saponins are more effective against gram positive and *in vitro* produced saponins are microbial against gram negative bacteria.

A number of workers have investigated the occurrence of antimicrobials active compounds from higher plants (Dhar et al. 1973; Atal et al. 1978; Dhawan et al. 1977, 1980; Aswal et al. 1984 a, b). Aderotimi and Adeyemo (2006) carried out phytochemical screening and antimicrobial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium* and determined the antimicrobial activity of three plant extracts contained saponins, tannins and alkaloids. According to Fluck, 1973 Saponins are a special class of glycosides which have soapy characteristics. It has also been shown that saponins are active antifungal agents (Sodipo et al. 1991). This therefore supports the earlier finding that extracts of the plants used in the present work may be useful in the chemotherapy of microbial infections.

**Table 1:** Callus Growth of *Convolvulus pluricaulis*

| Basal Media | PGRs Supplement | | Growth of *C. pluricaulis* | Appearance of callus |
|---|---|---|---|---|
| | 2,4-D (mg/l) | Kinetin (mg/l) | | |
| MS | 1 | 1 | +++++ | Whitish green, quite compact |
| | 2 | 2 | +++ | Whitish green, Compact |
| | 3 | 3 | +++ | Green, friable |
| | 4 | 4 | ++ | Dark green Friable |
| | 5 | 5 | + | light green, friable, granular embryonic |

**Table-2** Comparison of *in vivo* and *in vitro* produced Saponins of *C. pluricaulis* through HPTLC (resolved at 200 nm)

**Table-3** Comparison of *in vivo* and *in vitro* produced Saponins of *C. pluricaulis* through HPTLC (resolved at 254 nm)

| Spot No. | Rf | *C. pluricaulis* | |
|---|---|---|---|
| | | *in vivo* | *in vitro* |
| 1 | 0.01 | P | P |
| 2 | 0.05 | P | |
| 3 | 0.12 | P | P |
| 4 | 0.20 | P | |
| 5 | 0.22 | | P |
| 6 | 0.24 | P | P |
| 7 | 0.32 | P | P |
| 8 | 0.33 | P | P |
| 9 | 0.47 | P | P |
| 10 | 0.61 | P | |
| 11 | 0.64 | P | P |
| 12 | 0.80 | | P |
| 13 | 0.91 | P | |

| Spot No. | Rf | *C. pluricaulis* | |
|---|---|---|---|
| | | *in vivo* | *in vitro* |
| 1 | 0.04 | P | |
| 2 | 0.13 | | P |
| 3 | 0.17 | P | P |
| 4 | 0.20 | P | |
| 5 | 0.24 | P | P |
| 6 | 0.26 | | P |
| 7 | 0.35 | | P |
| 8 | 0.51 | | P |
| 9 | 0.64 | P | P |
| 10 | 0.76 | | P |
| 11 | 0.91 | P | P |

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