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Short Communication

Antimitotic Effect of Ethanol Fraction of *Hibiscus mutabilis* Leaf and Flowers

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***Hibiscus mutabilis* is reported for bioactive constituent. Ethanol fraction of *Hibiscus mutabilis* leaf and flower were studied for antimitotic activity. *Allium cepa* roots were used to evaluate cytotoxicity effect of the extracts. Ethanol soluble part of leaf and flower showed inhibitory effect on root length and root number growth on third day of incubation which is comparable with paracetamol as standard drug used. Reduction in number of dividing cells in the root meristem due to ethanolic fraction of leaf reveals antimitotic activity which is dose dependant. The constituents present in ethanolic extract may be responsible for cytoskeleton or tubulin polymerization or degradation that demonstrates antimitotic behavior.**

Key words: Antimitotic, *Hibiscus mutabilis*, cytotoxicity, *Allium sepa*, Ethanolic fraction

Hibiscus mutabilis is native to southern China and is favorite landscape plant in mild winter climates (The Wealth of India, 2001). *Hibiscus mutabilis*, also known as the Confederate rose or the cotton rosemallow. The most notable characteristic of this flowering shrub is that flowers are of three distinct colors appear on the bush simultaneously as the blooms color cycle independent of one another (Ishikura, 1982). Leaves and roots are said to be edible part of this plant. The flowers are of three distinct colors appear on the bush simultaneously as the blooms color cycle independent of one another. It shows white colour after flowering and gradually changes to red due to storage of anthocynin in petal vacuole, as cyaniding glycosedes have been isolated from deep red petals. Rutin has been reported in leaves whereas root is edible and fiber and mucilages are without very much flavor (Duke et. al, 1885). Fukui et al., (1971) isolated free cyanidin in flowers of *Hibiscus mutabilis*. Lowry, (1976) identified floral flavonol and anthocyanins in some malesian *Hibiscus* species. Free cyanidin in flowers of *Hibiscus mutabilis* was studied by Lowry (1971). This may be the first unequivocal case of free anthocyanidin occurring in flowers. In present study we have made an attempt to investigate the antimitotic effect of *Hibiscus mutabilis* leaf and flower by in vitro model of *Allium cepa* root test.

Materials and Methods

Collection and identification of the plant

The plant specimens for the proposed study were collected in morning in the month of Oct. 2008 from Sangamner area in

Ahmednagar District (M.S.). India. The herbarium was deposited at Botanical survey of India, Pune, for authentication and Dr. Mujumdar authenticated the plant as *Hibiscus mutabilis* Linn. belonging to family Malvaceae.

Extraction of plant material

Dried and coarsely powdered leaf and flowers are fractionated with ethanol by macerating for three days. It is then filtered and concentrated. The concentrated extracts were dried under vacuum to form dried ethanol fraction of flower (EF) and dried ethanol fraction of Leaf (EL).

Preparation of solution

Dried fractions were weighed for preparation of sample solutions in the concentration of 25 mg/ml and 50 mg/ml solution. Paracetamol is taken as standard in the concentration of 300ug/ml for positive control whereas tap water is used as negative control.

Anti-mitotic activity

Genotoxic activity was analyzed using the *Allium cepa* test. Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with the extracts and control. The test tubes were kept in an incubator at 24 ± 2 0C and the test samples were changed daily. After 72 h the roots were counted and their lengths were measured for each onion. When the newly emerged roots measured 2.0 − 3.0 cm, they were fixed. The fixative was glacial acetic acid/absolute alcohol (1/3 v/v). The root tips were kept in the ethyl alcohol- acetic acid (3:1) solution for 24 h. After fixation, the roots were transferred to 70% ethyl-alcohol and stored in refrigerator. For examination, the root tips were put into a watch glass to which 9 drops of aceto-orcein and 1 drop of 1 M HCl were added and warmed over a flame of spirit lamp for 2-3 min. These were kept at room temperature for 20 min. After removing the root caps from well-stained root tips, 1 mm of the mitotic zones were immersed in a drop of 45% acetic-acid on a clean slide and squashed under a cover glass. Mitotic index (MI), total cells and dividing cells were the cytological parameters studied, MI were expressed in terms of divided cells/total cells. Statistical analysis was performed on the collected data. The means of the control and extracts were obtained from descriptive analysis **(**Zmen O Ali et. al., 2007**)**.

Results and Discussion

Root number and root length measured in control and the extract solutions are shown in table 1 as well as Mitotic index are shown in table 2.

Table 1: Average root length and number in sample groups after 72 hrs.

Group	Root numbers Mean± SD	Root length (mm) Mean± SD
Control	24.0±3.2	26.1±2.2
Standard (300ug/ml)	06.5±2.0	02.5±4.1
EF (25 mg/ml)	20.0±3.1	14.3±3.7
EF (50 mg/ml)	18.0±1.4	09.0±2.2
EL (25 mg/ml)	12.4±3.3	08.4±3.1
EL (50 mg/ml)	05.4±4.0	03.0±1.2

EF is the ethanol extract of flower and EL is ethanol extract of leaf

Table 2. Mitotic index (MI) of different sample groups

Group	MI (Percent)
Control	8.6
Standard (300ug/ml)	2.4
EF (25 mg/ml)	9.2
EF (50 mg/ml)	7.5
EL (25 mg/ml)	5.3
EL (50 mg/ml)	3.1

Hibiscus mutabilis Linn. Leaf extracts reduced root number and root length when compared with flower extracts. In conformity with animal and human cell cytotoxicity it is found that extracts have cytotoxic properties also in plant test system (Swami et al., 2000 and Thabrew et al., 2005). This extracts are more effective on

root length and number when compared with the other extracts. It is evident that all extracts reduced the mitotic index. Leaf also showed less mitotic index compare to flowers and hence we can say that leaf extracts showed anti mitotic activity which is dose dependant. This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatments as expressed by growth inhibition of the roots of onion bulbs. It has been reported that the results from *Allium* test fit in well in a test battery composed of procaryotes and /or other eucaryotes (Fiskesjo et al., 1993, Fiskesjo, 1994). *Hibiscus mutabilis* contains presence of flavonoids and phenolic compounds (Chang, 2003). These compounds are reported to possess anticancer potential (Masataka et al., 2002). These types of compounds are present in leaf and flower of the plant. But the study indicates that leaf extracts are more active than flower extracts. Hence the leaf extract may contain antimitotic constituents that can stop the mitosis in anywhere of the cell cycle (Grant, 1982).

Conclusion

Ethanol soluble group of chemical constituents present leaf as well as flowers of *Hibiscus mutabilis* L. may be responsible for in vitro activity. These constituents probably affect the cytoskeleton or tubulin polymerization or degradation.

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