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RESEARCH ARTICLE

COMPARISON OF ANTI-MITOTIC AND CYTOTOXIC POTENTIAL OF *PIPER CHABA* (CHAVAK) AGAINST VINCRISTINE BY USING GERMINATION INHIBITION AND *ALLIUM CEPA* ROOT TIP ASSAY.

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Abstract

In the present study, we used *Vigna radiata* germination inhibition and *Allium cepa* root tip assay to demonstrate the Anti-mitotic and cytotoxic potential of *Piper chaba* (Chavak) aqueous extract. We compared the effectiveness of the extract with known anti-cancer drugs, vincristine. We observed nearly complete germination inhibition by the plant extract in the *Vigna radiata* assay. Furthermore, mitotic indices were significantly reduced compared to control. Our study revealed very effective antimitotic as well as cytotoxic activity of the *Piper chaba* aqueous extract like the vincristine.

Introduction:-

Recent reports are suggesting alarming conditions about the life-threatening disease which is undoubted; Cancer. Around 15 percent of deaths worldwide are attributed to cancer. Worldwide, Cancer causes about one-fifth of the deaths in the United States each year. By 2020, about nine hundred thousand Indians are expected to affect by the disease. Not only these in some nations, cancer have also crossed the number of heart disease and became the most common cause of death(Smith, Andrews et al. 2017).

It is also unlikely that there will ever be a single cure for cancer, as "cancer" refers to a class of diseases and a variety of etiological factors are associated with it. There are many types of cancer treatments available depending upon the type of cancer and its advancement. Mostly a combination of treatments is chosen in the combat of cancer such as surgery with chemotherapy or radiotherapy. Stem cell transplant, laser treatment, and photodynamic therapy are some of the most advanced methods applied to treat cancer. Apart from these, we also face the problem of the side effects caused by synthetic drugs and resistance against chemotherapeutic agents our general preference is now diverted towards the herbal medicine, with over more than a half of the world population using medicinal plants for the basic medical treatments (Cordell 1995).

One of the major groups of medicinal importance to treat cancer till date is Anti-mitotic agents. Primarily Antimitotic drugs like Vinca alkaloids and Taxol family target microtubules and show cytotoxic effects lead to great success in the cancer therapeutics. (McGrogan, Gilmartin et al. 2008). In general, Anti-mitotic agents affect the cell division of neoplastic cells and also the normal cells leading to more complicated clinical conditions like myelosuppression (Rowinsky, Eisenhauer et al. 1993).

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Despite major scientific and technological progress in combinatorial chemistry and structure-based design, natural products remain an important source of information on how to create compounds that can modulate new biological targets (Farnsworth 1988), (Cragg, Newman et al. 1997, Shoeb 2006). Green plants, in general, are the primary source of naturally occurring toxins and functioning against mutagenic agents (Gichner, Velemínský et al. 1993) and can be screened for antimitotic activity of the plant by using *Vigna radiata (V. radiata)* seed germination assay (Murthy, Francis et al. 2011). *Allium cepa* root tip meristematic cells has proved to be a reliable and cost-effective system for the rapid detection of cytotoxic and mutagenic properties (Abhanga, Jogleker et al. 1991), (Latha and Panikkar 1999) (Kowalczyk, Krzesiński et al. 2006).

The anti-mitotic and cytotoxic activity of plants have been observed by researchers to quote few examples; *Lavandula stoechas* (Sultan and Çelik 2009), *Capparis spinosa* (Çelik and Aslantürk 2009), *Vernonia amigdalina (*Adegbite and Sanyaolu 2009*) Azadirachta indica*, *Morinda lucida*, *Cymbopogon citrates*, *Mangifera indica*, *Carica papaya* (Akinboro and Bakare 2007)etc. as well as *Rauvolfia tetraphylla* (Kavitha K. R. 2016). Antiproliferative and cytotoxic activity of *Piper chaba (P. chaba or commonly;* Chavak) is evaluated in the present work, using *V. radiata* seed germination inhibition assay and *Allium cepa* root tip assay. *P. chaba* is found throughout India, and other warmer regions of Asian countries which include Malaysia, Sri Lanka, and Indonesia. It is a creeper plant that spreads on the ground. It may also grow around large trees. It is commonly used as a spice and an important constituent of certain recipes. Roots of this plant is mainly used for its strong aroma. In this study, we evaluated the antimitotic and cytotoxic potential of its aqueous extract using *V. radiata* and *Allium* root tip assay.

Materials and Methods:-

Collection and preparation of aqueous extract for *Vigna radiata* assay:

Lyophilized powder of root part of the plant was purchased from a certified dealer. 100 mg / mL plant material was used extract was prepared in water and then centrifuged at 10 K for 15 minutes. A clear supernatant was used for the study.

The *Vigna radiata* germination assay:

The *Vigna radiata* seeds (n = 10) of almost equal size and dry weight were selected for the assay. The seeds were pre-treated with water (washed and soaked) and were brought up to a stage where they are about to germinate. Such seeds were then imbibed in the control solutions or plant extracts. A set of 10 seeds were kept for each control and plant extracts. Seeds were incubated in microtiter plate with one seed in each well having 0.5 ml of control solutions and plant extract. The seeds were allowed to incubate for 72 hrs. Sprout length was recorded as a parameter to check the cytotoxic effect of the extract.

Collection and preparation of aqueous extract for *Allium* test:

Lyophilized powder of root part of the plant was purchased from a certified dealer and 20 mg/ml and 40 mg/ml aqueous extracts were prepared and centrifuged at 10000 rpm for 15 minutes. The clear supernatant was used for the assay.

***Allium cepa* root tip assay:**

The test was performed according to the method described by Fiskesjo, 1985. Onions (*Allium cepa*) were grown in water under laboratory conditions (light, 24ºC). Onions having root length of approximately 5cm were incubated in 20 mg/ml and 40 mg/ml aqueous extracts. We have used four to six root tips for each concentration from analogous onions then fixed using Carnoy's Fixative (3:1 v/v mixture of Ethanol and Glacial acetic acid), macerated using HCl and stained with Acetocarmine stain.

Mounting and slide observation:

Root-tips were taken on a clean glass slide, squashed in a drop of glacial acetic acid, covered with a coverslip and observed under the "Motic" microscope (40X Magnification) having a camera and software attachment (Motic Live Imaging Module). Photographs of several fields were captured. Chromosome morphology and their changes were observed and the mitotic index was calculated. Five onions in each group in triplicates were used for the analysis of the data. Mitotic Index (%) was calculated using this formula: No. of Dividing Cells x 100 / Total No. of cells

The controls of the Experiments:

Positive control: Aqueous Vincristine (200 µg / ml)
Negative control: Water

Statistical analysis:-

The data represented were analyzed by student t-test using Sigma Stat 2.0 statistical analysis software. The normality of data was tested by the Shapiro-Wilk test prior to the Student t-test. p values *p≤0.05 was considered as statistically significant.

Results:-

Results of our study have clearly pointed out that the *Piper chaba* aqueous extract treated seeds were unable to germinate while healthy sprout were observed in the water control group. The cytotoxic effect of the extract was found as significant as vincristine (*p≤0.05) (fig.-1.1 and 1.2). These results were further confirmed by using *Allium* test. Plant aqueous extract in two variable concentrations; 20 mg/ml and 40 mg/ml was able to reduce the mitotic index of root tips in the *Allium* test. Cells were found arrested in the prophase and chromosomes of the cells were condensed which indicated Anti-mitotic and cytotoxic activity of the extract. This significant reduction in the mitotic index compared to the control group was the same as the vincristine treated group indicated the strong Anti-mitotic and cytotoxic potential of the *Piper chaba* extract (fig.-2.1 and 2.2).

Discussion:-

We used plant-based model systems to check the Antimitotic and cytotoxic effect of *Piper chaba* aqueous extract *in vitro* by *Vigna radiata* germination inhibition assay and *Allium cepa* root tip assay. Our findings were compared with both positive and negative control groups. We used water as a negative control group in which normal growth of *Vigna radiata* seeds as well as normal cell division in meristematic cells of *Allium* bulbs was observed. Potent Antimitotic agent which is also a cancer chemotherapeutic agent *vincristine* was used as positive control and its effect was compared to the effect of *Piper chaba* extract. Vincristine has significantly reduced the germination of the seeds and Mitotic index compared to the negative control group. The aqueous extract treated seeds had shown the cytotoxic effect and resulted into the same inhibition of germination as that of vincristine. *Allium* bulbs treated with extracts have shown prophase arrest. This antimitotic effect may be due to compound/s present in the extract affecting chromosomal morphology or maybe to the spindle orientation. Our study indicated that the *Piper chaba* extracts possess cytotoxic activity and may contain anticancer metabolites which can be further evaluated by various advanced methods.

Conflict of Interest:

We declare no conflict of interest

Acknowledgment:-

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Figure 1.1:-*Vigna radiata* germination inhibition assay: Images of seeds (n=10) incubated for 72 hrs. Healthy sprouts of *V.radiata* were observed in negative control (a) Inhibition of seed germination in positive control by Vincristine-200µg/ml (b) Inhibition of germination by *piper chaba* extract (c).

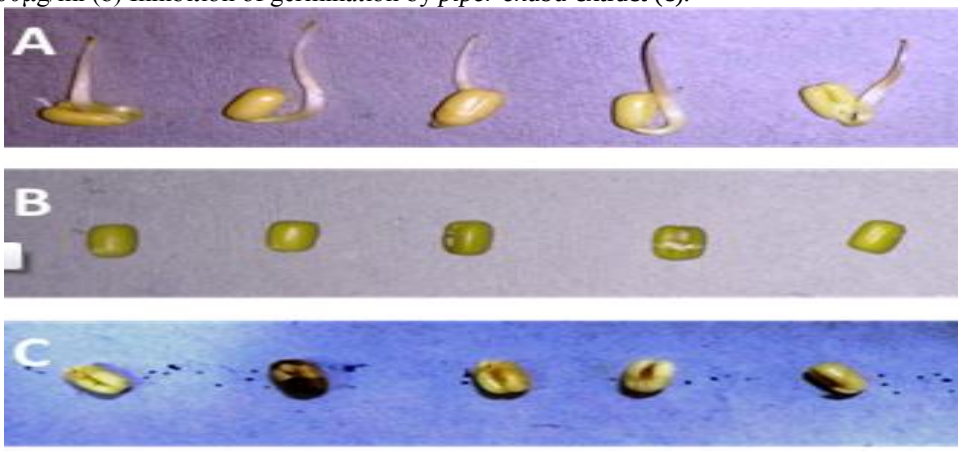


Figure 1.2:-Comparison of the potential of germination inhibition: Compared to water control, aqueous extract of *Piper chaba* had shown the significant (*p≤0.05) reduction in seed germination, indicative of cytotoxic/antimitotic potential of plant extracts

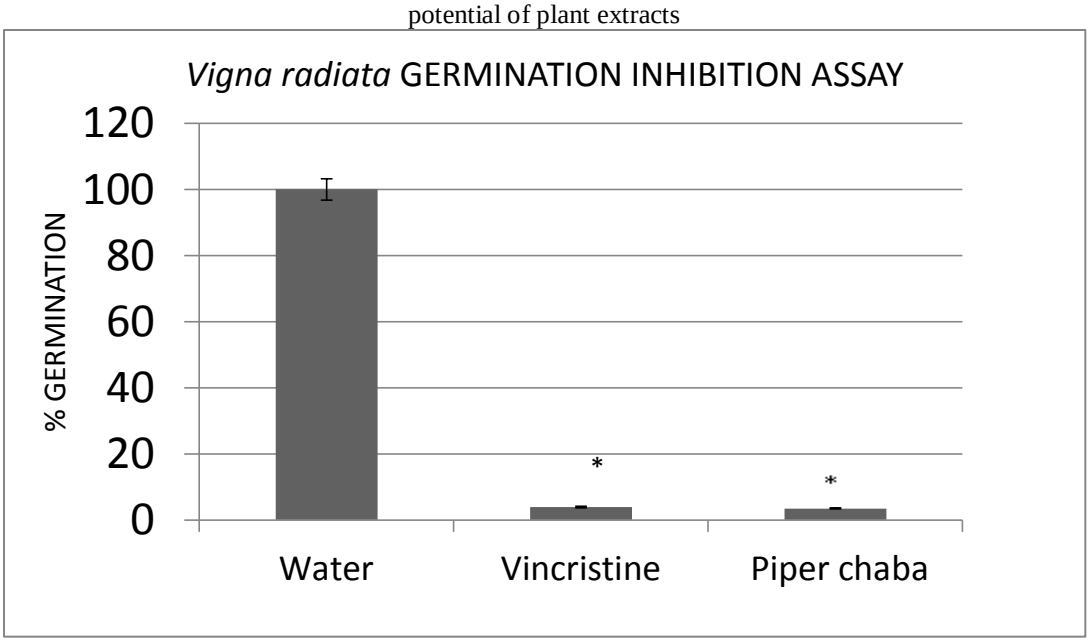


Figure 2.1:-Microscopic images of Allium cepa root tip assay: Normal well defined mitotic cell division in *Allium* root tip meristematic cells (A) Prophase arrest and cytotoxicity in vincristine treated tips (B) *Piper chaba* aqueous extract (20 mg/ml and 40 mg/ml) treated tips shown prophase arrest and cytotoxicity (C-D)

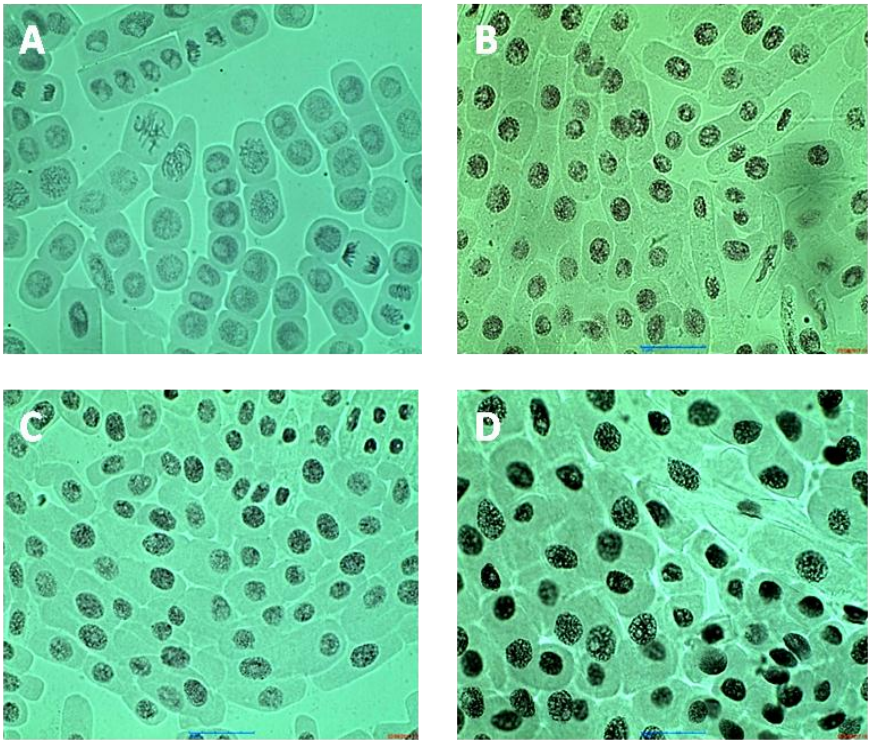
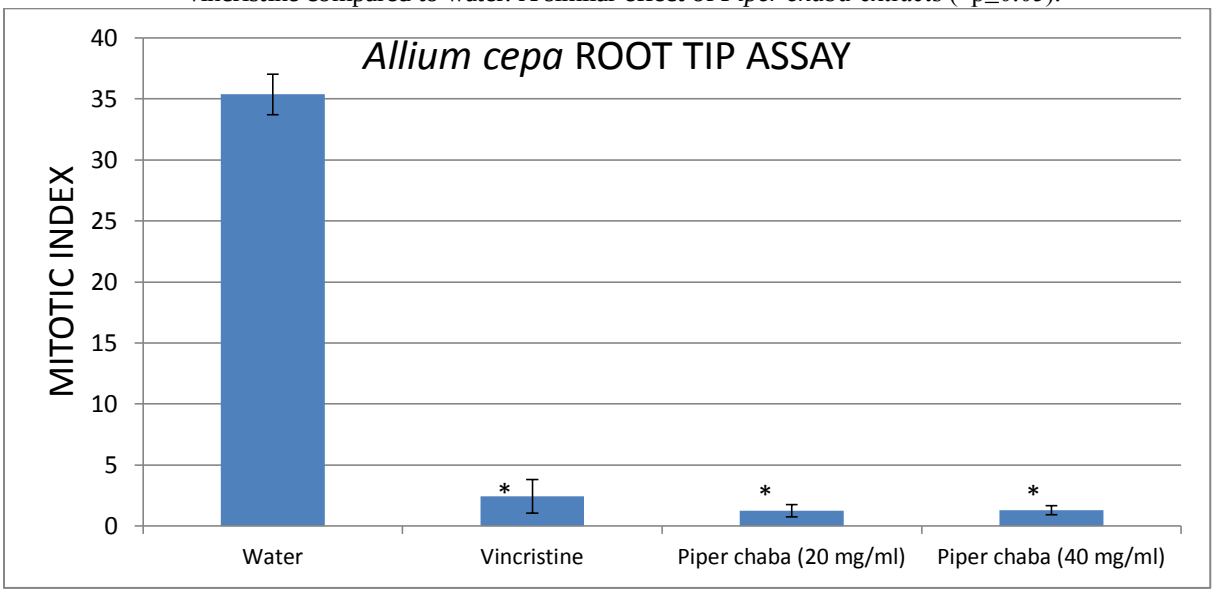


Figure 2.2:- Mitotic index based comparison of antimitotic potential: Significant reduction in mitotic index by vincristine compared to water. A similar effect of *Piper chaba* extracts (*p≤0.05).



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