

Chapter 5

Evaluation of phytotoxic, antioxidant and anticancer activities of *Parthenium hysterophorus* L. and *Cannabis sativa* L.

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Abstract: Medicinal plants contain variety of phytochemicals, including lignans, terpenoids, alkaloids, flavonoids, phenolic acids, etc. with a variety of biological functions. In the present study, *Cannabis sativa* and *Parthenium hysterophorus* are investigated for phytotoxic, antioxidant and anticancer activity along with their phytochemical analysis. Results demonstrated that aqueous extracts of selected plants leaves had significant phytotoxic effect on growth of *Vigna radiata* L (Mung bean) and *Zea mays* L. (Maize) seedlings as evident from various parameters studied (shoot length, root length, fresh weight and dry weight). Results of antioxidant study revealed that both aqueous extracts (*C. sativa* & *P. hysterophorus* leaves) showed good scavenging activities. The IC₅₀ value were found to be 348.21 µg/ml and 610.50 µg/ml respectively. Further, both extracts also showed antiproliferative effects against human non-small cell lung carcinoma (NSCLC) A549 in MTT assay. The GI₅₀ for aqueous extracts of *C. sativa* & *P. hysterophorus* leaves were found to be 47.75 µg/ml and 53.78 µg/ml respectively. Activities obtained in the present studies are may be due to various phenolic phytochemicals present in the extracts. Conclusively, results of the present study highlight bioactive efficacy of both plants selected plants.

Keywords: *Cannabis sativa*, *Parthenium hysterophorus*, Phytotoxic, Antioxidant, Antiproliferative

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Introduction: Over the course of human history, plant resources have remained a crucial component of civilization. Mankind has looked for an appropriate medicine among plants to treat a variety of ailments (WHO 2002; Mouele et al. 2022). Since the dawn of human civilization, medicinal plants have served as a source of healing in local communities throughout the world. Nevertheless, it continues to be crucial in today's world as a primary healthcare method for over 85% of the world's population (Pesic 2015) and as a source for drug discovery, since 80% of all synthetic pharmaceuticals come from plant (Bauer and Bronstrup 2014). The annual market value for medicinal plant products surpasses \$100 billion worldwide (Sofowora et al. 2013). Various wild plant products are a regular source of income for millions of people in underdeveloped nations (Maroyi 2017). Traditional medicinal systems have made considerable use of ethnomedicinal plants to treat a variety of diseases (El-Seedi et al. 2013). This connection dates back to the Neanderthal man, who treated illnesses using plants. The world society has acknowledged that many indigenous groups rely on biological resources, particularly medicinal plants, despite the fact that they are very old (Akerele 1993). Due to their widespread availability, cultural acceptance, and challenging economic circumstances, traditional medicinal plant cures the majority of the world's population (Hamilton 2004). 25% of prescription pharmaceuticals come from higher plants, whereas 75% of herbal medications were generated via study on traditional medicinal plants (Wang et al. 2002). The vast majority of bioactive chemicals with a variety of therapeutic qualities are found in medicinal plants. Anti-inflammatory, antiviral, anticancer, antimalarial, and analgesic qualities are only a few of the numerous therapeutic benefits linked to medicinal plants (Negi et al. 2011; Aye et al. 2019). Western Ghats and the Himalayas are two of India's most biodiverse areas, making it one of the world's wealthiest countries in terms of plant species. Out of the 43,000 plant species that exist in the nation, over 7500 are included in different medicines, and 1700 species are recognized in the Ayurvedic literature. Phytochemicals are utilised in India for a variety of purposes besides medicine, including cosmetics, health and hygiene, scent, and dietary supplements (Singh et al. 2020). Among various medicinal plants, *Cannabis sativa* and *Parthenium hysterophorus* are important medicinal plant with immense therapeutic properties. Both are regarded as weed in India. In the present study, *Cannabis sativa* and *Parthenium hysterophorus* are investigated

for phytotoxic, cytotoxic and antioxidant activity along with their phytochemical analysis.

Methodology: Leaves of *C. sativa* and *P. hysterophorus* were collected from the campus of SD College Barnala, Punjab (India) in the month of April. After the collection, material was washed 4-5 times in running water followed by grinding in the presence of distilled water with a help of pestle and mortar. Extract was filtered in conical flask with Whatman filter paper. The filtered extracts (Liquid) were used for phytotoxic studies. For the antioxidant and anticancer activity, this filtered extract so obtained was concentrated using Rotary Vacuum Evaporator and dried. The extracts were named as follows: Aqueous extract of *C. sativa* as WCS and aqueous extract of *P. hysterophorus* as WPH.

Phytochemical Screening (Harborne 1998)

Phenolics: Few drops of ferric chloride were added to the extract solution. The formation of dark green colour indicated the presence of phenolic acids.

Flavonoids: NaOH (1N) was added to the extract solution. Mixture turns yellow in colour, followed by addition of dil. HCl, solution turns colourless indicating presence of flavonoids.

Phytotoxic Studies: Liquid extract of leaves of *C. sativa* (WCS) and *P. hysterophorus* (WPH) were evaluated for their phytotoxic activity by measuring growth parameters of *Vigna radiata* L (Mung bean) and *Zea mays* L. (Maize) seedlings respectively. Seed viability of both *V. radiata* (Mung bean) and *Z. mays* (Maize) was tested employing 2,3,5 triphenyl tetrazolium chloride test.

2,3,5- Triphenyl Tetrazolium Chloride Dye Test: In this experiment about 100 seeds of selected plants were soaked overnight in water and then their seed coats were removed to expose seeds. The seeds are cut into halves in such a way that seeds cut exactly into half along with an embryo. Then these cut seeds are treated with 0.5 % of 2,3,5- triphenyl tetrazolium chloride dye for about half an hour in dark. The seed turns reds if they are viable while no viable seeds remain colourless.

Treatment of *V. radiata* (Mung bean) seeds with liquid extract of WCS: Seeds of *V. radiata* were soaked overnight and then placed in the glass petriplates containing wet cotton. After one day, seeds were treated with liquid extract of WCS and allow to grow for seven days. After seven days, growth parameters such as shoot length, root length, fresh weight and dry weight were measured.

Treatment of *Z. mays* (Maize) seed with liquid extract of WPH

Seeds of *Z. mays* were soaked overnight and then placed in the glass petriplates containing wet cotton. After one day, seeds were treated with liquid extract of WPH and allow to grow for seven days. After seven days, growth parameters such as shoot length, root length, fresh weight and dry weight were measured.

Antioxidant Activity: Little modifications were made to the procedure given by Blois (1958) for the DPPH radical scavenging test. Extracts of varying concentrations (300 µl) were added to a clean test tube followed by addition of 2 ml of a 0.1 mM methanolic DPPH solution. The solution was mixed by shaking and incubated in dark place for next 30 minutes for the completion of

scavenging reaction. The final absorbance was read at 517 nm. Rutin was used as a standard antioxidant.

DPPH radical scavenging potential of extracts was calculated using formula:

$$\text{DPPH radical scavenging activity (\%)} = Z_0 - Z_1 / Z_0 \times 100$$

where,

Z_0 is the absorbance of control solution

Z_1 is the absorbance of reaction mixture containing test sample

Anticancer Activity: Cytotoxic activity of both extracts was assessed against Human lung cancer (A549) cells. Cell line was purchased from the National Centre for Cell Science (NCCS), Pune, India. In DMEM media containing 10% foetal bovine serum and an antibiotic/antimycotic solution, A549 lung cancer cells were grown and cultured. Trypan blue dye was added to the cells to check their viability. Only the cells that were still unstained by the dye were considered to be viable. For cytotoxicity investigations, the cell population with viability of more than 95% was employed.

MTT assay: The cytotoxic potential of extracts of selected plants was assessed using MTT assay (Mosmann 1983) with slight modifications. For the experiment, cells were seeded into 96 well plate at the density of 8000 cells per well and allowed to adhere. After complete adherence of cells, cells were treated with various concentrations of extracts for next 24 h. After treatment, cells were treated with MTT for next 3 hrs. After that media was carefully removed from wells of 96 well plates and 100 μ l DMSO was added to each well to dissolve formazan crystals. The final absorbance of solution was taken at 570 nm using ELISA plate reader (Synergy HT BIOTEK).

Cytotoxicity of extracts was calculated using formula:

$$\text{Cytotoxic activity (\%)} = Z_0 - Z_1 / Z_0 \times 100$$

where,

Z_0 is the absorbance of control cells

Z_1 is the absorbance of cells treated with extracts

Statistical Analysis: The results were showed as average $\pm$ standard error. Regression analysis was done using best fit method. GI_{50}/IC_{50} values (50% inhibitory concentrations) were calculated with help of regression equations. One-Way ANOVA analysis was also done followed by determination of honestly significant difference (HSD) among means using Tukey's test. The significance of results was checked at $p \leq 0.05$ or 0.001.

Results and Discussion: Qualitative phytochemical analysis showed presence of phenolics in WCS and WPH extract while flavonoids were detected in WCS only. In the current study, both extracts viz. WCS and WPH were evaluated for their phytotoxic effect on the *V. radiata* (Mung bean) and *Z. mays* (Maize) seedlings growth respectively. The seeds of *V. radiata* (Mung bean) and *Z. mays* (Maize) showed 100% seed viability in Tetrazolium seed viability test. WCS extract significantly inhibited the growth of shoot and root length of 7- days old Mung seedlings. Extract showed shoot length of 2.66 ± 0.088 cm at highest tested concentration of 10% (Figure 1). Control showed shoot length of 16.2 ± 1.665 cm. WCS also inhibited the root length (0.83 ± 0.233) at highest tested concentration

as compared to the control seedlings (3.46 ± 0.481 cm). WPH extract significantly reduced the growth of shoot and root length of 7- days old maize seedlings. The shoot length was found to be 5.50 ± 1.041 cm at 5% concentration as compared to the control (13.23 ± 1.468 cm) (Figure 2). Extract also significantly decreased the root growth (8.1 ± 1.973 cm) as compared to the control (2.83 ± 0.667 cm). The treatment with extracts viz. WCS and WPH significantly reduced the parameters such as fresh and dry weight (Figure 3 & 4). As the concentrations of WCS extract increased, there was significant reduction in the fresh weight of Mung seedlings from 0.407 ± 0.052 g (control) to 0.057 ± 0.003 g at highest tested dose. There was reduction of 0.027 ± 0.003 g (highest tested concentration of 10 %) from 0.057 ± 0.003 g (control) in dry weight (Figure 3 & 4). On the other hand, WPH reduced the fresh weight of maize seedlings from 0.417 ± 0.011 g (control) to 0.248 ± 0.020 g (highest tested concentration of 5%). Seedlings also showed dry weight of 0.150 ± 0.025 g at highest tested concentration of 5% as compared to control (0.020 ± 0.012 g) (Figure 3 & 4). Similar results were found in the studies of Khan et al. (2021), they reported phytotoxic effects of medicinal plant, *Justicia adhatoda* L. on germination and growth of cauliflower, broccoli, tomato, foxtail millet and barnyard grass under laboratory and in pot conditions. Results revealed that high extract concentrations decreased germination and growth (shoot length, root length, and biomass weight) of seedlings as compared to control. Effects of aqueous extracts of Eucalyptus (*Eucalyptus camaldulensis*) and sugar beet (*Beta vulgaris*) on the germination and growth of purslane (*Portulaca oleracea*) was studied by Dadkhah et al. (2013). The results demonstrated that the concentration of water extract from the examined plants did not affect the germination percentage of purslane seeds. However, aqueous extracts of the investigated plants profoundly impacted seed vigour index and seedling growth.

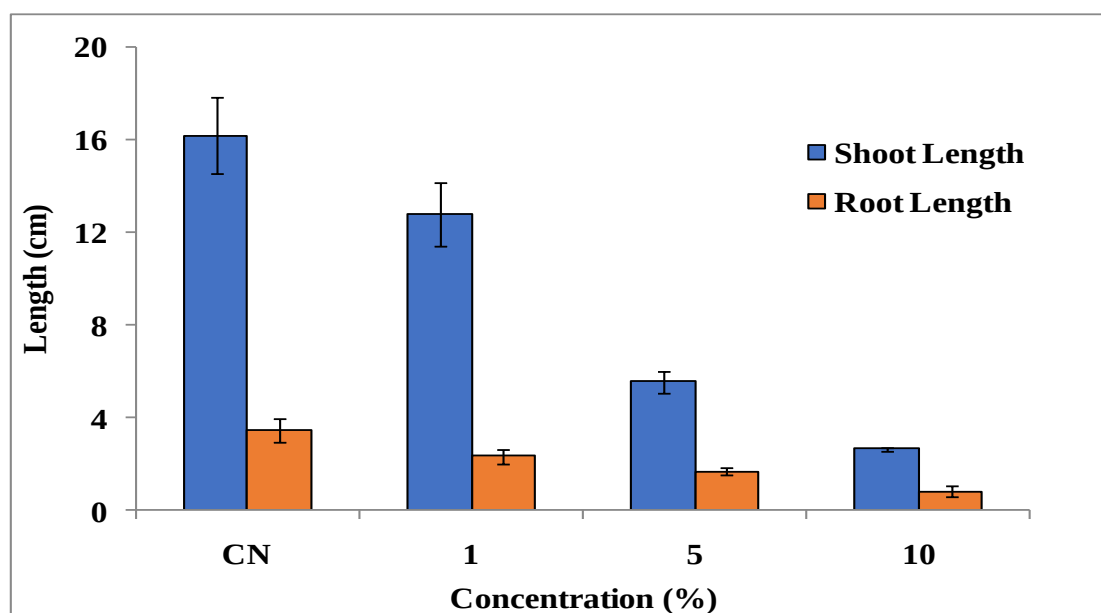


Figure 1: Effect of WCS extract on the shoot and root length of *V. radiata* (Mung bean)

[One way Anova analysis: F-ratio (3, 8): 32.105*; HSD: 5.00 (shoot length); F-ratio (3, 8): 11.79*; HSD: 1.46 (root length) (*p<0.05)]

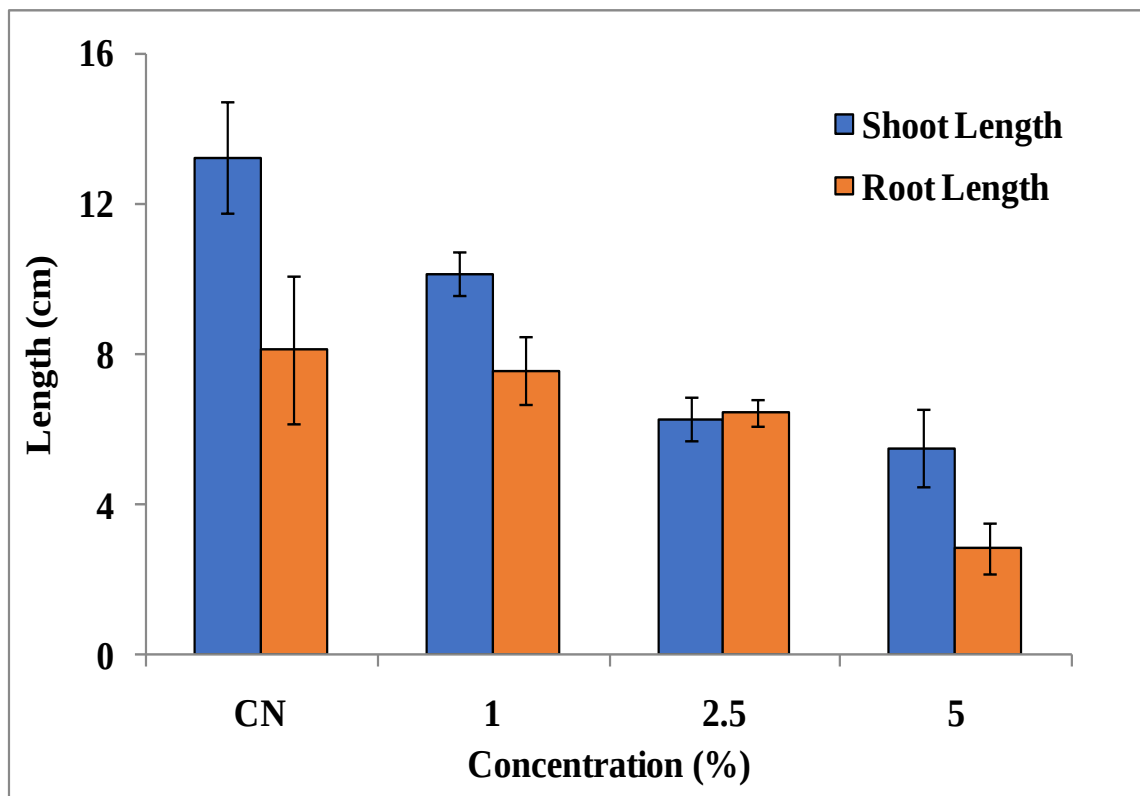


Figure 2: Effect of WPH extract on the shoot and root length of *Z. mays* (Maize)
[One-way Anova analysis: F-ratio (3, 8): 13.15*; HSD: 4.48 (shoot length); F-ratio (3, 8): 4.23*; HSD: 5.21 (root length) (*p≤0.05)]

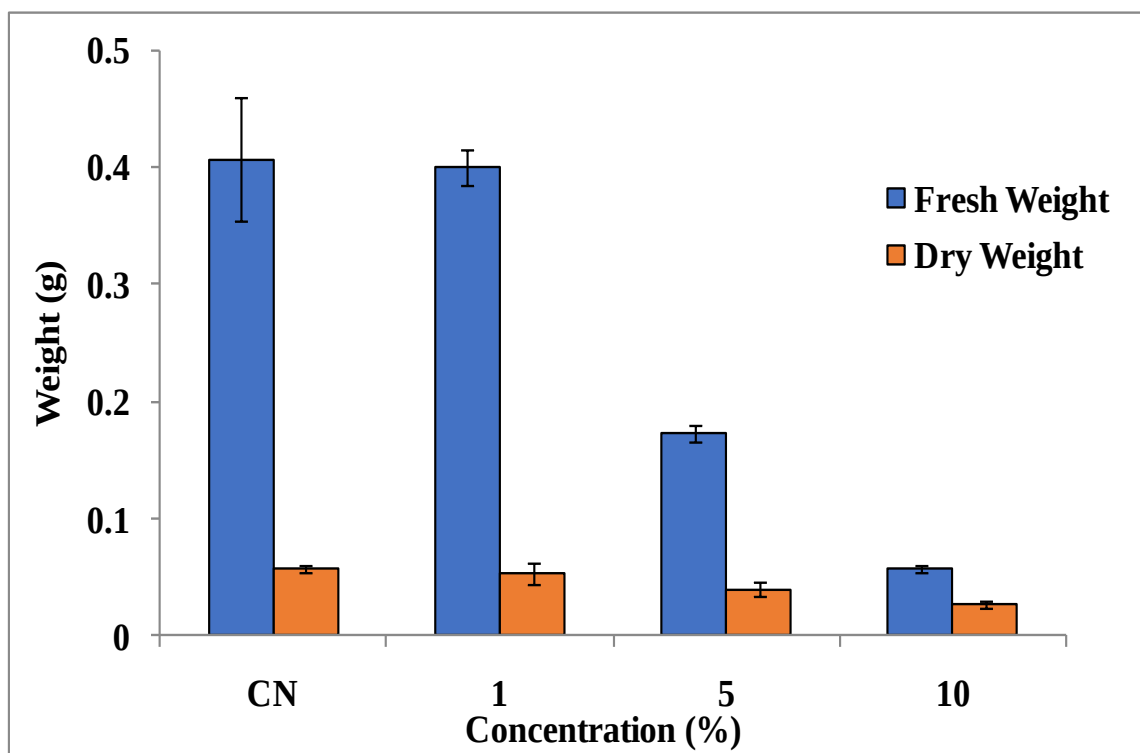


Figure 3: Effect of WCS extract on the fresh and dry weight of *V. radiata* (Mung bean)
[One-way Anova analysis: F-ratio (3, 8): 22.63*; HSD: 0.16 (fresh weight); F-ratio (3, 8): 5.61*; HSD: 0.02 (Dry weight) (*p≤0.05)]

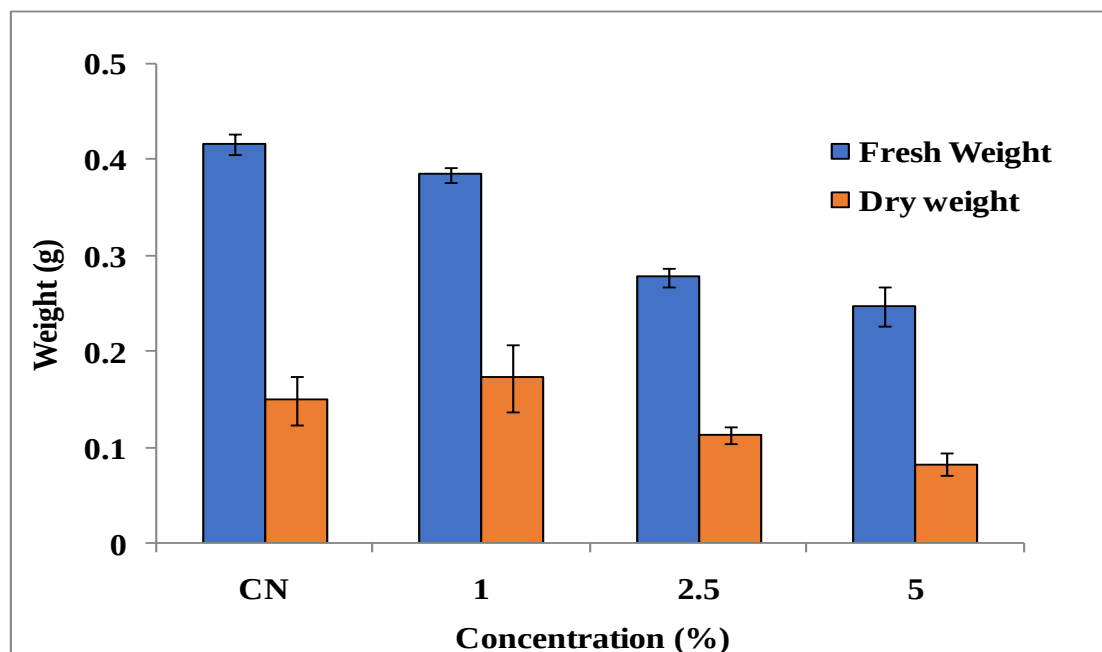


Figure 4: Effect of WPH extract on the fresh and dry weight of *Z. mays* (Maize)

[One-way Anova analysis: F-ratio (3, 8): 37.55*; HSD: 0.06 (fresh weight); F-ratio (3, 8): 3.00; HSD: 0.10 (Dry weight) (* $p \leq 0.05$)]

Ramalakshmi et al. (2015) investigated phytotoxicity of several organic extracts from the leaves of the medicinal plant *Tabebuia rosea* (TR) on radish seeds. At 10,000 ppm, all TR extracts dramatically reduced root growth. However, the petroleum ether and ethanol fractions had greater germination rates for radish seeds.

Antioxidant studies: WCS extract significantly scavenged the DPPH free radicals in the DPPH assay. As evident from the results, extract showed inhibition of 76.72 % at highest tested dose of 1000 $\mu\text{g/ml}$. The lowest tested concentration *i.e* 50 $\mu\text{g/ml}$ exhibited inhibition of 11.98%. The IC_{50} was found to be 348.21 $\mu\text{g/ml}$. Results were compared with standard antioxidant rutin (IC_{50} 43.39 $\mu\text{g/ml}$) (Figure 5). WPH extract demonstrated inhibition of 64.60 % at highest tested concentration of 1000 $\mu\text{g/ml}$. The lowest tested concentration *i.e* 50 $\mu\text{g/ml}$ showed inhibition of 4.54 %. The IC_{50} was found to be 610.50 $\mu\text{g/ml}$ (Figure 6). Antioxidant efficacy of extracts is may due to presence various phenolic compounds as detected in phytochemical analysis. Results were compared with standard antioxidant rutin (IC_{50} 43.39 $\mu\text{g/ml}$). Similar results were reported by various workers on different medicinal plants, Kumar et al. (2011) reported antioxidant activity of chloroform and ethyl acetate fractions of *Koelreuteria paniculata* using DPPH assay along with other experiments. Results demonstrated that extract possessed high antioxidant efficacy as compare to control. Authors correlated the activities with phenolic and flavonoid compounds in the extracts. In another study, Tailor et al. (2014) reported DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of different extracts of aerial parts like leaves and flowers of *Ageratum conyzoides* Linn. Plant, Extracts showed high antioxidant activity with an IC_{50} value of 9.3 and 24.8 $\mu\text{g/ml}$ for ascorbic acid and alcoholic leaves extract respectively. High

antioxidant potency of extracts could be because of its phenolic and flavonoid contents.

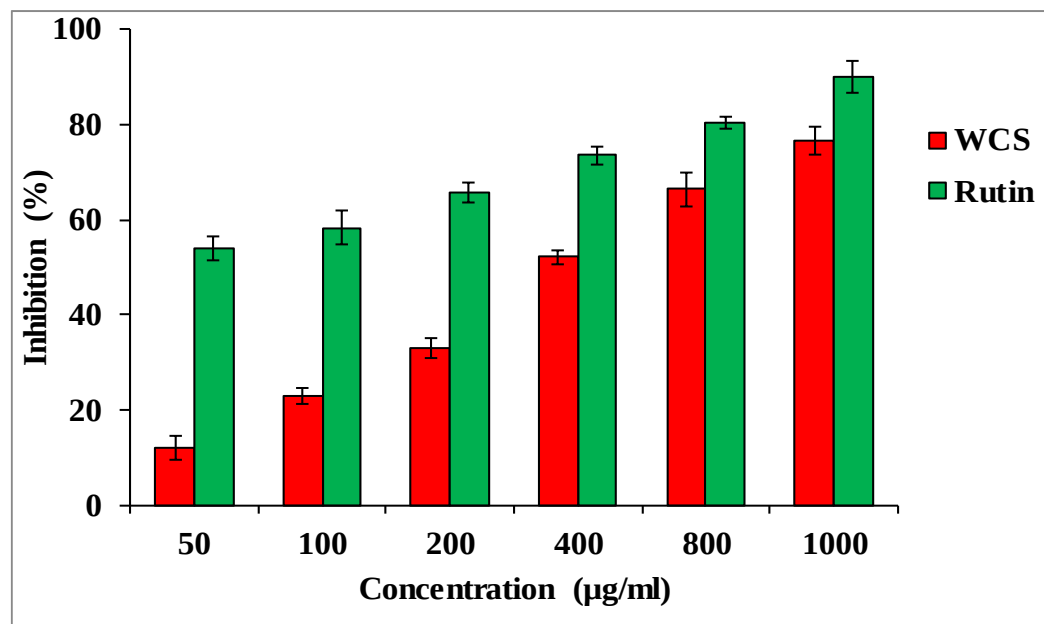


Figure 5: Antioxidant activity of WCS and standard rutin in DPPH radical scavenging assay
[One-way Anova analysis: F-ratio (5, 12): 98.77; HSD: 12.13 (*p≤0.001)]

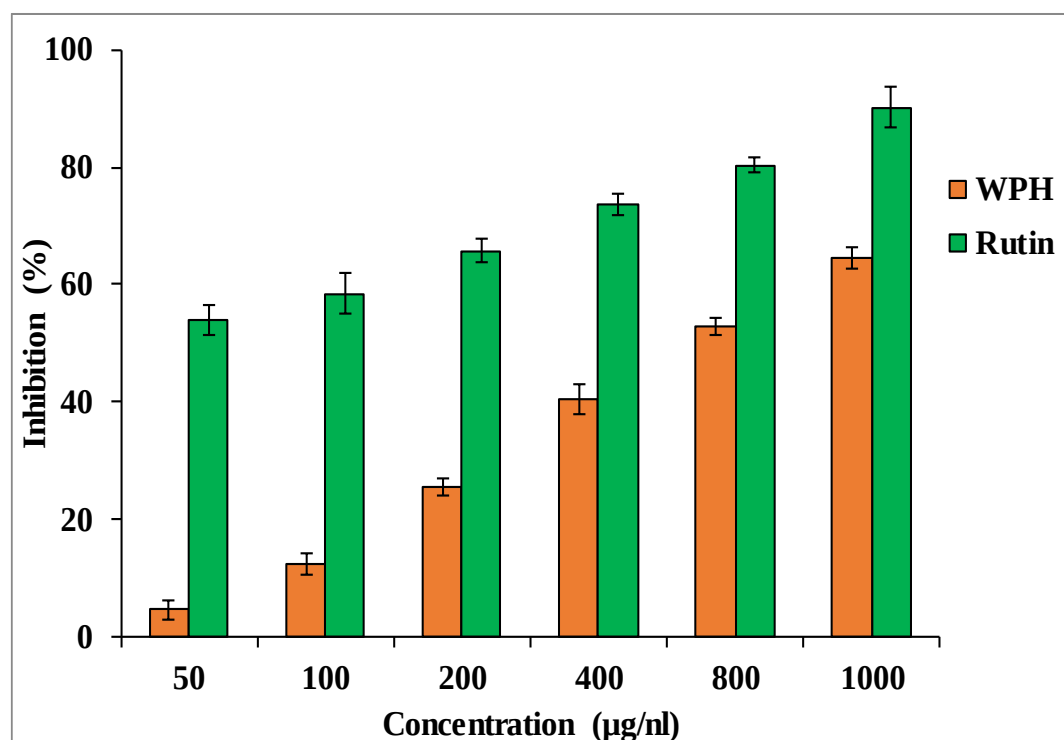


Figure 6: Antioxidant activity of WPH and standard rutin in DPPH radical scavenging assay
[One-way Anova analysis: F-ratio (5, 12): 163.67; HSD: 8.69 (*p≤0.001)]

Anticancer studies: The anticancer effects of both extracts *viz.* WCS and WPH were evaluated against human non-small cell lung carcinoma (NSCLC) A549 in MTT assay. Results showed cytotoxicity of 16.84 % and 10.88 % for WCS and

WPH extracts respectively at lowest tested concentration of 50 µg/ml. The highest tested concentration of 1000 µg/ml showed cytotoxicity of 92.67 and 88.60 % respectively (Figure 7). The GI₅₀ for WCS and WPH was found to be 47.75 µg/ml and 53.78 µg/ml respectively. Extracts activity was due to presence of phytoconstituents as detected in phytochemical analysis. Similar results were reported by various workers. The cytotoxic activity of *Tecoma stans* extracts was compared with that of the drug vincristine against a lung cancer cell line (Robinson et al. 2017). Results indicated that there was a significant increase in cell death with an increase in extract concentration. The SRB test was used to assess the anticancer activity of leaf extracts from 10 *Ipomoea* species on the A549 cell line and the HepG2 cells (Rane and Patel 2015). The crude aqueous extracts *Ipomoea* species showed significant antiproliferative activity against HEPG2 and A549 cells. For the HEPG2 cell line, *I. cairica* was the most effective growth inhibitor (GI₅₀ at 30.6 µg/ml), followed by *I. hederifolia* (GI₅₀ at 38.1 µg/ml) and *I. carnea* (GI₅₀ at 40.0 µg/ml). Out of different species, only *I. aquatic* showed significant results with a GI₅₀ at 75.6 µg/ml against A549 cell line.

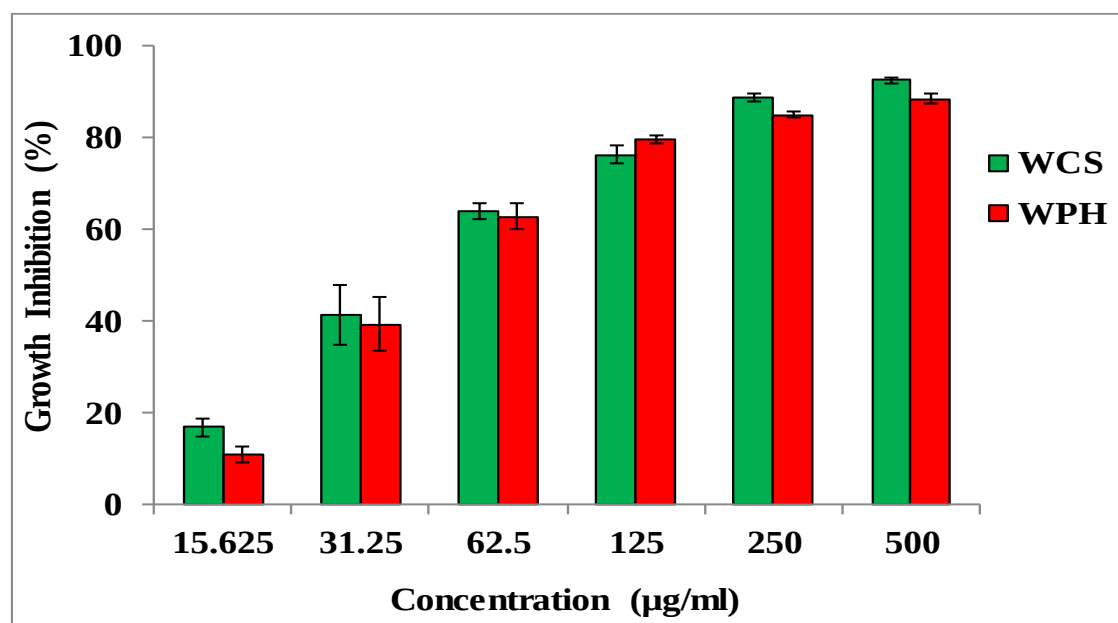


Figure 7: Growth inhibitory effects of various concentrations of WCS and WPH on A549 Lung cancer cells in MTT Assay

[One-way Anova analysis: F-ratio (5, 12): 91.41; HSD:14.61 (WCS); F-ratio (5,12): 113.90; HSD:13.60 (WPH) (*p<0.001)]

Conclusion: Results of the present study demonstrated that extracts of both plants viz. *C. sativa* and *P. hysterophorus* possess phytotoxic, antioxidant and antiproliferative activity. Further studies are needed to isolate and identify various phytochemicals and their mode of action.

Conflict of authors: The authors have no conflicts of interest regarding this investigation

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