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# ALLELOPATHIC POTENTIALITIES OF AQUEOUS LEAF EXTRACTS OF *OCHLANDRA WIGHTII* (MUNRO) C.E.C. FISCH. IN GAMBLE ON GERMINATION AND BIOCHEMICAL PARAMETERS OF *CICER ARIETINUM* (L.)

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## ABSTRACT

*The aqueous extracts of fresh and dry leaves of Ochlandra wightii showed both inhibitory and stimulatory effects on seed germination, Biochemical and Phytochemical components in Cicer arietinum (Bengal gram) seedlings. The different parameters were found to be either decreasing or increasing on adding the aqueous leaf extracts of different concentrations from 1% to 9%. The maximum germination was shown in control condition and the minimum or no growth was reported in 10%, 20%, 50% and 100%. The seeds germinated at concentration below 10% were taken for different analysis. The biochemical and phytochemical constituents in the shoot and leaf and in the cotyledon of the germinated seeds of Cicer arietinum were also analysed. The pigment analysis of the leaves was also undertaken. These results indicate that the inhibitory effect on the germination of seeds may be due to allelopathy and the allelochemicals present in the leaves of this bamboo species. The findings of the present investigation showed that the stimulatory and inhibitory effect of O. wightii aqueous extracts of fresh and dry leaves may be due to the presence of allelochemicals in the leaves of bamboo species.*

## KEY WORDS

*Ochlandra wightii, Cicer arietinum, Allelopathy.*

## INTRODUCTION

Allelopathy refers to both inhibitory and stimulatory reciprocal biochemical interactions between all types of plants and includes the addition of a chemical compound to the environment. It is the release of chemical compounds into the environment by one plant and these chemical compounds give a direct or indirect harmful effect on another plant (Rice 1974) [1]. Culpeper (1633) [2] found that basil and rye never grow together. He also found that there is an antipathy between grape and cabbage plants. Bonner and Galston (1944) [3] found that other plants do not grow near Parthenium due the presence of a toxin, trans-cinnamic acid in their root exudates. Not only the root exudates but also leaves play a role in allelopathy as in walnut the inhibitory products are released from the shed leaves to the surroundings. Stickney and Hoy (1881) [4] found that the vegetation under black walnut (*Juglans nigra*) was very sparse and crops did not grow near this plant. According to Bode (1958) [5], allelopathic effect of walnut was due to the leaching of a bound toxin from the walnut leaves, stems and branches, which underwent hydrolysis and oxidation in the soil and form true toxin and killed the neighbouring plants. Bode (1940) [6] and Funke (1943) [7] found that Artemisia

leaves release inhibitory products. The leaves of guayule produce benzaldehyde which has inhibitory effects (Bonner 1946) [8]. When the leaves of *Pinus densiflora* are washed by rain, the neighbouring plants become badly affected (Lee and Monsi 1963) [9]. *Salvia leucophylla* and *Artemisia californica* produce some terpene like compounds (Harborne, 1977) [10] which strongly inhibit the surrounding vegetation and as a result bare patches are formed. *Leucaena leucocephala* contains a toxic component in leaves that inhibits the growth of other trees but not its own seedlings. Leucaena species have been reported to reduce the yield of wheat but this species increases the yield of rice. Many botanists, farmers and gardeners have suggested many cases of allelopathy for over 2000 years. Controlled scientific experiments on allelopathy were not conducted until after the year 1900. Several studies have been conducted on the ecological, environmental and sustainable uses of reed bamboos. There are several studies based on the allelopathic effect of some bamboos but there isn't any systematic study related to allelopathy of the reed bamboos. There are different types of secondary metabolites and biologically active compounds that can be isolated and used in pharmacognosy or in agriculture.

## MATERIALS AND METHODS

The fresh and dry leaves of *O. wightii* were collected from the reserve forest areas of Kallar, Thiruvanathapuram, Kerala. 100 gms of fresh and dry leaves of *O.wightii* were mixed with 500 ml of distilled water and ground fine, so that aqueous extracts were obtained. This solution was the stock solution (100%) and different concentrations, 10%, 20% and 50% were made from this stock solution by adding distilled water. Apart from this, concentrations from 1% to 9% were also made. Cotton was spread on Petri plates and sample seeds of *Cicer arietinum* were sown on it. Each Petri plate was labelled. The aqueous extracts of different concentrations were applied to each Petri plates everyday and water was given for control plates. These Petri plates were kept at $25^0$ C growth chambers and analysed for 10 days. The rate of seed germination was noted in all concentrations and the control Petri plates every day.

The various biochemical constituents analyzed include Carbohydrates (Hedge& Hofreiter, 1962), Protein (Lowry *et. al*., 1951), Starch (Hedge & Hofreiter ,1962), Reducing sugar (Miller, 1959), Aminoacid (Moore and Stein ,1948), Fatty acid( Cox and Peaeson,1962), Total Phenolics (Malick and Sigh, 1980), Tannin (Robert, 1971), Saponin (Fenwick and Oakenfull 1983), Alkaloid and Flavanoid (Spectrophotometer),Oxalate (Oxalate assay buffer), resin(liquid nitrogen), Vitamin C (Harris and Ray,1935), Vitamin A (Baker *et. al.,*1980), Nutrient Dietry fibre and its components (Enzymatic gravimetric method,1992), Iron (AAS). The moisture content was determined using ISTA method (1996), leaf pigments (spectrophotometer).

## RESULT AND DISCUSSION

In *O. wightii*, the value of carbohydratre, protein, reducing sugars, amino acids, dietry fibres, phenols, flavanoids, alkaloids and saponin content for the case of different concentrations of fresh and dry leaf extract were analysed in both shoots and in cotyledon of the seedlings of *Cicer arietinum*. Different Vitamins, iron, starch, moisture content, tannin, fatty acid, oxalate, resin and some pigments were also analysed in the cotyledons. The present investigation was carried out with 10%, 20%, 50% and 100% concentrations of fresh and dry leaf extracts of *O. wightii*. But the seeds of *C. arietinum* did not show any response to these concentrations. Hence, further studies were continued with concentrations from 1 to 10%.

The analysis carried out with the fresh and dry leaf extract of *O. wightii* in the shoots and leaves and cotyledons of C. *arietinum* showed that the Carbohydrate, Protein, reducing sugar, amino acid and dietary fibre were decreasing as the concentration increased from1% to10%. But the values for phenol, flavonoids, alkaloids and saponin were found to be increasing in gram seeds treated with both fresh and dry leaf extracts in shoots and cotyledons as the concentration was increased from 1% to 10% except for flavonoids analysed in the leaves and shoots treated with fresh leaf extracts of *O. wightii*.(Table 1,2,5 & 6).

The different components like Vitamin A, iron, starch and moisture content in the cotyledons grown with the fresh and dry leaf extracts were found to be decreasing as the concentration increased. Oxalate and resin were directly proportional to the concentrations in cotyledons treated with both fresh and dry leaf extracts of *O. wightii*. The amount of fatty acid in the cotyledons treated with fresh leaf extract was increasing in concentrations from 1% to 10% whereas, it was found

to be decreasing when treated with dry leaf extract. The presence of tannin in the cotyledons treated with fresh leaf extract was inversely proportional when compared with the dry leaf extract where it was found to be increasing. (Table 3& 7). The Pigment analysis in the leaves of *C. arietinum* with fresh and dry leaf extract of *O.wightii* showed that all the pigments analysed were found to be inversely proportional to the concentration. (Table 4 & 8).

**Table 1: Chemical constituents and its variation in shoots of *Cicer arietinum* (fresh leaf extract of *O.wightii*)**

| Component | Control (mg/gm) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Carbohydrate*** | 13.45 | 13.23 | 12.78 | 12.34 | 11.94 | 11.76 | 11.23 | 10.01 | 9.33 | 8.55 |
| ***Protein*** | 6.34 | 6.44 | 6.35 | 6.21 | 6.11 | 5.74 | 5.50 | 5.48 | 4.81 | 4.42 |
| ***Reducing sugar*** | 5.27 | 7.59 | 7.12 | 6.37 | 6.33 | 5.34 | 5.22 | 5.04 | 4.98 | 3.30 |
| ***Aminoacid*** | 5.43 | 4.33 | 3.88 | 3.76 | 3.29 | 3.26 | 3.14 | 3.01 | 2.89 | 2.87 |
| ***Dietry fibre*** | 7.21 | 8.62 | 7.54 | 7.12 | 6.91 | 6.84 | 6.73 | 6.49 | 6.41 | 6.33 |
| ***Phenols*** | 1.11 | 0.87 | 1.25 | 1.29 | 1.43 | 1.46 | 1.56 | 1.65 | 1.73 | 1.79 |
| ***Flavanoids*** | 0.65 | 0.88 | 0.87 | 0.64 | 0.45 | 0.38 | 0.32 | 0.26 | 0.23 | 0.11 |
| ***Alkaloids*** | 0.56 | 0.03 | 0.04 | 0.05 | 0.06 | 0.07 | 0.07 | 0.08 | 0.08 | 0.09 |
| ***Saponin*** | 0.52 | 0.13 | 0.21 | 0.26 | 0.32 | 0.37 | 0.43 | 0.48 | 0.51 | 0.56 |

**Table 2: Chemical constituents and its variation in cotyledon of *Cicer arietinum* (fresh leaf extract of *O.wightii*)**

| Component | Control (mg/gm) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Carbohydrate*** | 10.67 | 15.63 | 14.71 | 13.77 | 13.45 | 13.13 | 12.62 | 12.48 | 11.63 | 11.33 |
| ***Protein*** | 3.86 | 7.60 | 7.49 | 6.39 | 6.11 | 5.62 | 5.34 | 5.01 | 4.86 | 4.32 |
| ***Reducing sugar*** | 6.34 | 9.56 | 8.95 | 8.23 | 8.10 | 7.39 | 6.63 | 5.22 | 4.96 | 4.28 |
| ***Aminoacid*** | 4.55 | 3.57 | 3.15 | 2.58 | 2.19 | 1.54 | 1.47 | 1.22 | 1.04 | 0.98 |
| ***Dietry fibre*** | 5.62 | 8.33 | 7.54 | 7.26 | 6.57 | 6.31 | 6.20 | 5.42 | 5.31 | 5.28 |
| ***Phenols*** | 0.54 | 0.33 | 0.65 | 0.97 | 1.16 | 1.23 | 1.65 | 1.72 | 1.78 | 1.82 |
| ***Flavanoids*** | 0.48 | 0.13 | 0.22 | 0.27 | 0.28 | 0.34 | 0.42 | 0.47 | 0.52 | 0.64 |
| ***Alkaloids*** | 0.41 | 0.03 | 0.04 | 0.05 | 0.07 | 0.08 | 0.09 | 0.11 | 0.14 | 0.17 |
| ***Saponin*** | 0.36 | 0.12 | 0.15 | 0.19 | 0.21 | 0.22 | 0.24 | 0.25 | 0.26 | 0.27 |

**Table 3: Various constituents and its variation in cotyledon of *Cicer arietinum* (fresh leaf extract of *O.wightii*)**

| Component | Contol (mg/gm) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Vitamin A*** | 0.41 | 1.45 | 1.36 | 1.11 | 0.76 | 0.48 | 0.27 | 0.22 | 0.19 | 0.16 |
| ***Vitamin C*** | 1.15 | 1.22 | 1.59 | 1.39 | 1.31 | 1.22 | 0.82 | 0.57 | 0.22 | 0.17 |
| ***Iron*** | 0.98 | 0.67 | 0.52 | 0.51 | 0.49 | 0.47 | 0.41 | 0.39 | 0.26 | 0.14 |
| ***Starch*** | 7.86 | 8.07 | 7.45 | 7.36 | 6.10 | 5.74 | 5.43 | 4.37 | 4.22 | 4.10 |
| ***Moisture content*** | 36% | 74% | 72% | 65% | 61% | 60% | 57% | 55% | 53% | 52% |
| ***Tannin*** | 0.32 | 1.09 | 1.00 | 0.85 | 0.78 | 0.56 | 0.47 | 0.34 | 0.23 | 0.11 |
| ***Fatty acid*** | 3.66 | 1.89 | 2.07 | 2.11 | 2.13 | 2.33 | 2.49 | 2.51 | 2.73 | 2.81 |
| ***Oxalate*** | 0.07 | 0.09 | 0.25 | 0.33 | 0.39 | 0.45 | 0.67 | 0.71 | 0.86 | 0.92 |
| ***Resin*** | 0.18 | 0.31 | 0.52 | 0.65 | 0.70 | 0.91 | 1.00 | 1.10 | 1.19 | 1.28 |

**Table 4: Pigment analysis in leaves of *Cicer arietinum* (fresh leaf extract of *O.wightii*)**

| Pigments | Control (µg/g) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Chlorophyll a*** | 14.16 | 10.31 | 10.7 | 9.52 | 9.24 | 8.45 | 8.14 | 7.53 | 6.27 | 6.11 |
| ***Chlorophyll b*** | 11.19 | 8.66 | 8.32 | 7.81 | 7.43 | 6.28 | 5.76 | 5.24 | 4.74 | 4.00 |
| ***Carotenoid*** | 2.67 | 3.52 | 3.41 | 2.90 | 2.63 | 1.91 | 1.57 | 1.15 | 0.96 | 0.64 |
| ***Xanthophyll*** | 1.76 | 2.51 | 2.43 | 2.17 | 2.03 | 1.95 | 1.86 | 1.62 | 1.41 | 1.09 |
| ***Phaeophytin*** | 2.79 | 2.97 | 2.56 | 2.48 | 2.41 | 2.12 | 1.93 | 1.60 | 1.16 | 1.04 |

**Table 5: Chemical constituents and its variation in shoots of *Cicer arietinum* (dry leaf extract of *O.wightii*)**

| Component | Control (mg/gm) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Carbohydrate*** | 13.45 | 11.16 | 11.08 | 10.34 | 10.18 | 9.57 | 9.23 | 8.64 | 8.12 | 7.76 |
| ***Protein*** | 6.34 | 6.36 | 5.39 | 5.36 | 4. 87 | 4.84 | 4.61 | 4.35 | 4.19 | 4.03 |
| ***Reducing sugar*** | 5.27 | 5.84 | 5.21 | 5.11 | 5.04 | 4.93 | 4.84 | 4.62 | 4.53 | 3.98 |
| ***Aminoacid*** | 5.43 | 4.78 | 3.33 | 3.25 | 3.18 | 3.09 | 2.97 | 2.87 | 2.71 | 2.09 |
| ***Dietry fibre*** | 7.21 | 7.30 | 7.11 | 6.96 | 6.82 | 6.47 | 6.36 | 6.25 | 6.18 | 5.91 |
| ***Phenols*** | 1.11 | 1.54 | 1.68 | 1.72 | 1.78 | 1.81 | 1.94 | 2.03 | 2.11 | 2.18 |
| ***Flavanoids*** | 0.65 | 0.38 | 0.43 | 0.53 | 0.59 | 0.67 | 0.74 | 0.82 | 0.88 | 0.94 |
| ***Alkaloids*** | 0.56 | 0.10 | 0.11 | 0.16 | 0.19 | 0.23 | 0.26 | 0.29 | 0.31 | 0.34 |
| ***Saponin*** | 0.52 | 0.16 | 0.19 | 0.23 | 0.32 | 0.33 | 0.39 | 0.45 | 0.49 | 0.51 |

**Table 6: Chemical constituents and its variation in cotyledon of *Cicer arietinum* (dry leaf extract of *O.wightii*)**

| Component | Control (mg/gm) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Carbohydrate*** | 10.67 | 13.27 | 12.83 | 12.06 | 11.13 | 11.02 | 10.88 | 10.63 | 10.39 | 10.03 |
| ***Protein*** | 3.86 | 6.37 | 6.23 | 5.27 | 5.16 | 5.15 | 5.03 | 4.43 | 4.27 | 4.07 |
| ***Reducing sugar*** | 6.34 | 9.45 | 8.56 | 8.08 | 7.97 | 7.45 | 7.16 | 7.02 | 6.93 | 6.43 |
| ***Aminoacid*** | 4.55 | 2.95 | 2.84 | 2.53 | 2.05 | 1.83 | 1.71 | 1.54 | 1.53 | 1.52 |
| ***Dietry fibre*** | 5.62 | 4.23 | 4.00 | 3.72 | 3.45 | 2.89 | 2.66 | 2.56 | 2.12 | 1.09 |
| ***Phenols*** | 0.54 | 0.56 | 0.73 | 1.25 | 1.52 | 1.64 | 1.87 | 1.92 | 1.99 | 2.06 |
| ***Flavanoids*** | 0.48 | 0.12 | 0.26 | 0.27 | 0.53 | 0.64 | 0.67 | 0.71 | 0.78 | 0.84 |
| ***Alkaloids*** | 0.41 | 0.06 | 0.09 | 0.13 | 0.18 | 0.24 | 0.27 | 0.29 | 0.31 | 0.34 |
| ***Saponin*** | 0.36 | 0.03 | 0.08 | 0.11 | 0.15 | 0.18 | 0.31 | 0.39 | 0.43 | 0.47 |

**Table 7: Various constituents and its variation in cotyledon of *Cicer arietinum* (dry leaf extract of *O.wightii*)**

| Component | Contol (mg/gm) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Vitamin A*** | 0.41 | 2.65 | 2.16 | 1.91 | 1.78 | 1.17 | 0.92 | 0.89 | 0.85 | 0.67 |
| ***Vitamin C*** | 1.15 | 2.37 | 2.12 | 1.94 | 1.62 | 1.49 | 1.21 | 0.94 | 0.72 | 0.64 |
| ***Iron*** | 0.98 | 1.77 | 1.43 | 1.29 | 0.96 | 0.85 | 0.72 | 0.54 | 0.41 | 0.37 |
| ***Starch*** | 7.86 | 6.84 | 6.45 | 6.17 | 5.94 | 5.82 | 5.18 | 4.94 | 4.64 | 4.28 |
| ***Moisture content*** | 36% | 63% | 62% | 57% | 56% | 55% | 54% | 52% | 51 % | 50% |
| ***Tannin*** | 0.32 | 0.98 | 1.11 | 1.12 | 1.15 | 1.17 | 1.21 | 1.24 | 1.29 | 1.34 |
| ***Fatty acid*** | 3.66 | 3.09 | 2.67 | 2.25 | 1.93 | 1.56 | 1.28 | 1.20 | 1.19 | 1.15 |
| ***Oxalate*** | 0.07 | 0.19 | 0.27 | 0.44 | 0.46 | 0.49 | 0.56 | 0.62 | 0.75 | 0.87 |
| ***Resin*** | 0.18 | 0.76 | 0.83 | 0.93 | 1.37 | 1.76 | 1.79 | 1.85 | 1.91 | 1.96 |

**Table 8: Pigment analysis in leaves of *Cicer arietinum* (dry leaf extract of *O.wightii*)**

| Pigments | Control (µg/g) | 1% | 2% | 3% | 4% | 5% | 6% | 7% | 8% | 9% |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Chlorophyll a*** | 14.16 | 8.65 | 8.67 | 7.53 | 7.43 | 7.37 | 7.24 | 7.17 | 7.09 | 6.32 |
| ***Chlorophyll b*** | 11.19 | 5.70 | 5.42 | 4.81 | 4.35 | 4.11 | 3.89 | 3. 62 | 3.39 | 3.0 |
| ***Carotenoid*** | 2.67 | 2.62 | 2.53 | 2.20 | 2.12 | 1.61 | 1.43 | 1.27 | 1.14 | 0.83 |
| ***Xanthophyll*** | 1.76 | 1.81 | 1.63 | 1.52 | 1.48 | 1.35 | 1.22 | 1.17 | 1.14 | 1.09 |
| ***Phaeophytin*** | 2.79 | 1.42 | 1.38 | 1.27 | 1.23 | 1.16 | 1.09 | 1.06 | 1.02 | 0.06 |

## CONCLUSION

The present study focuses on the allelopathic effect of *O. wightii* on *Cicer arietinum*. The aqueous extracts of both fresh and dry leaves of *O.wightii* are influencing the biochemical and phytochemical compounds in *Cicer arietinum*. The fresh and dry leaves of *O.wightii* contain several secondary metabolites like phenol, alkaloid, flavanoids, saponins etc. in considerable quantities which is either stimulating or inhibiting the growth of *Cicer arietinum*. The therapeutic values of this plant also justify it as a potential source of useful drugs. This is the first report on allelopathic effect for the fresh and dry leaves of *Ochlandra wightii.* Hence further studies related to the analysis, isolation, purification and characterisation of the bioactive compounds from the leaves of *O. wightii* is recommended.

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