

Original Research Article

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Phytochemical Composition of Aqueous Crude Extracts of Selected Pesticidal Plants used against *Brassica* Vegetable Pests

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ABSTRACT

The phytochemistry of five plants (*Phytolacca dodecandra*, *Azadirachta indica*, *Capsicum frutescens*, *Allium cepa* and *Tagetes minuta*) was determined to evaluate their potential as sources of alternative pesticides against *Brassica* vegetable pests. Crude aqueous plant extracts were prepared, on which quantitative and qualitative analysis were performed using standard methods. Tannins and alkaloids were found in all plants examined with their highest quantities obtained in *T. minuta* at 0.4494 mgml⁻¹ and 0.1560 mgml⁻¹ respectively. Steroids were highest in *C. frutescens* and *A. indica* at 2.2791 mgml⁻¹ each. Saponins were more in *P. dodecandra* (31.06%) and flavonoids were highest in *C. frutescens* (26.13%). It was evident that *C. frutescens*, *A. indica* and *T. minuta* had higher quantities of phytochemicals. This may be responsible for them being more effective pesticides against *Brassica* vegetable pests than *Allium cepa* and *P. dodecandra* which had lower quantities or absence of some phytochemicals.

Keywords

Aqueous Extracts,
Pesticidal Plants,
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Introduction

Agriculture has from time immemorial been faced with the destructive activities of numerous pests like insects, fungi and weeds leading to a radical decrease in yields. Owing to this, control measures to minimize their effects on crops are vital. For many years,

chemical pesticides have proved to be effective in controlling pests and hence boosting crop production so much that many farmers have abandoned cultural pest control methods and turned all their attention to chemical pesticides (Baidoo and Mochiah, 2016). However, these pesticides have been criticized for the negative impacts associated

with their use such as environmental contamination that transfers chemical residues along the food chain, development of pesticide resistance in some pests, increased health hazards to people, threats to biodiversity and/or contributing to genetic defects in subsequent generations, and increased unproductiveness of arable land (Baidoo *et al.*, 2012; Naqqash *et al.*, 2016).

In light of these developments, there has been a renewed interest in the use of botanicals, plant derived products, for crop protection over the last few decades (Devi and Gupta, 2000; Facknath, 2006; Ssekyewa *et al.*, 2008; Tewary *et al.*, 2005; Miresmailli and Isman, 2014). Generally, the use of botanical pesticides is more sustainable and has a lower environmental impact than synthetic pesticides (Devanand and Rani, 2008).

The phytochemicals from these plants are responsible for their pesticidal potentials. Phytochemicals are secondary metabolites, biosynthesized from primary metabolites, eliciting pharmacological or toxicological effects in man and animals (Bernhoft, 2010).

Complex mixtures of secondary compounds in plant extracts were reported to contribute to a great deal to synergism, which enhances the joint action of active compounds against pests and reduces the rate of resistance development (Jacobs *et al.*, 2015). Studies by several researchers such as De Geyter *et al.*, (2007) and Adeniyi *et al.*, (2010) have shown that the most important phytochemicals well known to confer pesticidal properties are alkaloids, terpenoids, steroids, phenolic compounds, saponins and tannins. This study was therefore, designed to determine and compare the phytochemical components of crude aqueous extracts of five selected plant species (*A. indica*, *C. frutescens*, *P. dodecandra*, *Allium cepa* and *T. minuta*) used against pests of *Brassica* vegetables. The

results obtained are key in making recommendations concerning the cultivation and domestication of certain species whose bioactive substances can be valued as pesticides.

Materials and Methods

Plant Collection

Plant leaves of *A. indica*, *T. minuta* and *P. dodecandra* and *C. frutescens* fruits were harvested from Katanda Sub-County, Rubirizi District in Western Uganda in January, 2019.

Allium cepa bulbs were purchased from the trading centers in the same sub-county. The plant samples were identified at the Department of Biology, Mbarara University of Science and Technology, Uganda.

Plant Aqueous Extract Preparation

The fresh plant samples were washed thoroughly with running tap water to remove dust, pollen and other particles. They were then ground using a mechanical grinder and the ground samples extracted with 1 liter of distilled water for 24 hours at ambient temperature. The aqueous extract was filtered, the residue discarded and the solvent evaporated in an oven for 5 days at 65° C to yield crude extracts. These were packed into small labeled bottles, well covered and kept in a pharmaceutical refrigerator. An amount of 2.0 g of each crude extract was weighed and dissolved in 20 mL of distilled water in separate labeled beakers to obtain the aqueous plant extracts used for phytochemical screening.

Phytochemical screening

This was done using chemicals of analytical grade following methods described by Trease and Evans (2009) and Kokate *et al.*, (2010).

Alkaloids

An amount of 2 mL of the plant extract was boiled with 2 mL of 2% sulphuric acid for two minutes, cooled then a few drops of Dragendorff's reagent (DR) added. A reddish brown precipitate was formed indicating the presence of alkaloids.

Saponins

A 2 mL amount of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing indicated the presence of saponins.

Flavonoids

The extract (2 mL) was mixed with 3 mL of 1% sodium hydroxide and dilute hydrochloric acid was added. The appearance of a yellow colouration indicated the presence of flavonoids.

Tannins

Exactly 2 mL of the extract was mixed with 4 drops of ferric chloride solution. Formation of a blue colour indicated the presence of hydrolysable tannins while the formation of a green colour indicated the presence of condensed tannins.

Steroids

To 2 ml of the extract was added 4 drops of acetic anhydride and the mixture boiled and cooled. 2 mL of concentrated sulphuric acid was added down the side of the tube. Formation of a brown ring at the junction of the two layers and the upper layer turning green indicated the presence of steroids.

Quantification of Phytochemicals

Analytical method for quantitative determination of the phytochemicals were

carried out according to Amadi, *et al.*,(2004); Ejikeme, *et al.*,(2014) and Obadoni and Ochuko (2001).

Alkaloids

The quantification of alkaloids was done using quinine sulphate stock solution (prepared by dissolving 0.1 g in 100 mL distilled water) as the standard solution. Several dilutions of the stock solution were made by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0 mL of the stock solution into separate test tubes and diluting to 10 mL volume with distilled water. To each test solution, 3 mL of DR (prepared by mixing 0.8 g bismuth nitrate pentahydrate in 40 mL distilled water and 10 mL glacial acetic acid, and a solution of 8.0 g of potassium iodide in 20 mL distilled water) were added followed by 3 mL of thiourea solution (prepared by dissolving 3.0 g in 100 mL distilled water). Using a spectrophotometer (UV 6705/Vis. with 1 cm UV quartz cell), the absorbance values of the test solutions formed were measured at a wave length of 435 nm against colorless reagent (distilled water) blanks. A calibration curve of absorbance versus concentration stock solution was plotted and found to be linear(Figure 1).

Exactly 10 mL of each plant extract (prepared by dissolving 0.1 g of crude sample in 100 mL of distilled water in a beaker) was pipetted into five separate labeled test tubes. To each, 3 mL of DR were added followed by 3 mL of thiourea solution. The absorbance was measured at 435 nm against the blank of distilled water. The obtained value of the absorbance was substituted in the linear equation of the calibration curve to get the concentration of the alkaloids in mgml^{-1} .

Tannins

Tannins were quantified using tannic acid stock solution (prepared by dissolving 0.1 g in

100 mL of distilled water) as the standard solution. Several dilutions of the stock solution were made by pipetting out 0.2, 0.4, 0.6, 0.8, and 1.0 mL stock solution into separate test tubes and diluting to 10 mL volume with distilled water. To each test solution, 3 mL of iron III chloride (prepared by dissolving 5.0 gin 100 mL of distilled water) was added. Using a spectrophotometer, the absorbance values of the test solutions formed were measured at a wave length of 550 nm against colorless reagent (distilled water) blanks. A calibration curve of absorbance versus concentration was also plotted (Figure 2).

A 10 mL amount of each plant extract (prepared by dissolving 0.1 g of crude sample in 100 mL of distilled water) was pipetted out into five separate labeled test tubes. To each, 3 mL of iron III chloride were added. The absorbance was measured at 550 nm against the blank of distilled water. The obtained value of the absorbance was substituted in the linear equation of the calibration curve to get the concentration of the tannins in mgml^{-1} .

Steroids

Steroids were quantified using steroid stock solution (prepared by dissolving 1.0 g in 100 mL of distilled water) as the standard. Several dilutions were made by pipetting out 0.6, 0.8, 1.0 and 1.2 mL of stock solution into separate test tubes and diluting to 10 mL volume with distilled water. To each test solution, 3 mL of acetic anhydride, 3.30%, was added followed by 3 mL of concentrated sulphuric acid, 98%. Using a spectrophotometer, the absorbance values of the test solutions formed were measured at a wave length of 513 nm against colorless reagent (distilled water) blanks. A calibration curve of absorbance versus concentration was plotted (Figure 3). Exactly 10 mL of each plant extract (prepared by dissolving 1.0 g of crude sample in 10 mL of

distilled water) was pipetted out into five separate labeled test tubes. To each, 3 mL of acetic anhydride was added followed by 3 mL of concentrated sulphuric acid. The absorbance was measured at 513 nm against the blank of distilled water. The obtained value of the absorbance was substituted in the linear equation of the calibration curve to get the concentration of the steroids in mgml^{-1} .

Flavonoids

Crude extracts (5.0 g) were weighed and dissolved in 20 mL of distilled water in a beaker. The mixture was filtered, forming the aqueous phase that was transferred into a 250 mL separating funnel to which 40 mL ethyl acetate (organic phase) was added. The two were vigorously shaken and partitioned successfully after which the organic phase was collected into a clean pre-weighed beaker whereas the aqueous phase was retained in the funnel. To the aqueous phase was added 40 mL of n-butanol, the layers were vigorously shaken and partitioned. The aqueous phase was discarded while the organic phase was recovered and combined with the ethyl acetate extract. The samples were dried in the oven for 7 days at 65°C and thereafter weighed and flavonoid yield was calculated as a percentage using the equation below.

Flavonoid yield

$$= \frac{\text{mass of crude flavonoids}}{\text{mass of dry aqueous extracts}} \times 100\%$$

Saponins

Crude extract (12.5 g) were weighed and dissolved in 30 mL of distilled water in a beaker forming a uniform mixture that was the aqueous phase. This was transferred into a 250 mL separating funnel to which 20 mL diethyl ether (organic phase) was added to de-fat the extract. The two were vigorously shaken and partitioned successfully, the aqueous phase

was retained in the funnel whereas the organic phase was discarded. To the aqueous phase was then added 30 mL of n-butanol to extract the saponins, the layers were vigorously shaken and partitioned. Thereafter the aqueous phase was discarded while the organic phase was retained in the funnel. To the organic phase, 100 mL of 5% sodium chloride was added to wash out impurities in the n-butanol. The liquids were shaken vigorously and partitioned and then the n-butanol phase was collected in a clean dry pre-weighed beaker. The beaker was labeled and dried in the oven at 65°C to a constant weight. The saponin yield was calculated as a percentage using the equation below:

$$\text{Saponin yield} = \frac{\text{mass of crude saponin}}{\text{mass of dry aqueous extracts}} \times 100\%$$

Data Analysis

Means of triplicate analyses were calculated and data was expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) with Post hoc (Tukey HSD) statistical analysis was performed using SPSS Version 22 software for comparison between two or more treatments. A difference in ANOVA was considered to be statistically significant when $p \leq 0.05$.

Results and Discussion

Percentage yield of aqueous extracts of the pesticidal plants

Table 1 shows the percentage yield of aqueous extract of the plants. The crude extract of the *C. frutescens* fruit contains a greater proportion by mass of the component compounds followed by that of the *Allium cepa* bulbs. The leaf extracts of *T. minuta*, *A. indica* and *P. dodecandra* contains the least proportion of the component compounds respectively.

Phytochemical components of the pesticidal plants

The phytochemicals detected were alkaloids, saponins, flavonoids, tannins, and steroids as shown in Table 2. The results showed that all plants contained alkaloids and tannins. Steroids were absent in only *P. dodecandra* and flavonoids absent in *T. minuta*. Saponins were present only in *P. dodecandra* and *C. frutescens*.

Phytochemical Quantification

Table 3 shows the quantitative estimates of the phytochemicals in the plant extracts and their comparison using ANOVA, F test. There were highly significant differences in the mean concentrations of the phytochemicals across the five plant extracts ($p = 0.000$). Alkaloids and tannins were highest in *T. minuta* extract at $0.1556 \pm 0.000 \text{ mgml}^{-1}$ and $0.4494 \pm 0.027 \text{ mgml}^{-1}$ respectively. The same phytochemicals were lowest in *Allium cepa* extracts at $0.0871 \pm 0.001 \text{ mgml}^{-1}$ and $0.0407 \pm 0.002 \text{ mgml}^{-1}$ respectively. *C. frutescens* and *A. indica* extracts gave the highest concentrations of steroids each at 2.2791 mgml^{-1} and the least in *Allium cepa* at $0.5248 \pm 0.017 \text{ mgml}^{-1}$. Flavonoids were most in *C. frutescens* at $26.134 \pm 0.058\%$ and least in *P. dodecandra* at $12.134 \pm 0.006\%$. Saponins were found in *P. dodecandra* and *C. frutescens* and were estimated at $31.059 \pm 0.062\%$ and $7.786 \pm 0.058\%$ respectively. In a few cases, concentrations of some phytochemicals between plant extracts were statistically equal namely: alkaloid and flavonoid concentrations in *A. indica* and *Allium cepa* extracts with p-values of 0.1 and 0.759 respectively; also, tannin and steroid concentrations of *A. indica* and *C. frutescens* extracts with p-values of 0.995 and 1.0 respectively. Phytochemicals are the major source of pharmaceuticals, food additives, fragrances, pesticides, and herbicides (Okwu, 2005; Ramawat and Dass, 2009). However,

isolation of the pure, active constituents from plants for these purposes is a long and tedious process. Therefore, it is necessary to have methods available which eliminate unnecessary separation procedures such as chemical screening. Ndam *et al.*, (2014) states that this method is performed to allow localization and targeted isolation of new or useful constituents with potential activities. The authors continue to state that this procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important.

In the present study, phytochemical analysis was performed on aqueous plant extracts of *A. indica*, *T. minuta*, *Allium cepa*, *C. frutescens* and *P. dodecandra*. The phytochemicals, alkaloids, saponins, flavonoids, tannins, and steroids, were found to be present in the pesticidal plants in different proportions. The phytochemicals, when ingested by animals, exhibit various pharmacological and biochemical actions and hence may be responsible for their pesticidal properties. This concurs with related studies done by De Geyter *et al.*, (2007) in which it was highlighted that secondary metabolites such as phenolic compounds, saponins, alkaloids, flavonoids and terpenoids have been identified to exhibit strong activities against several pathogens and insect pests.

Phenolic compounds are one of the largest groups of plant metabolites (Singh, *et al.*, 2007) and owing to this, intensive studies have been undertaken with regard to their toxicity (Goławska, 2006). Halkier (1999) reports that phenols play important roles in plant herbivore and pathogen interactions. The antioxidant properties of pesticidal plants have been described by various studies to be linked to presence of phenolic compounds (Krings &

Berger, 2001). They usually produce natural oxidants such as flavonoids, phenolic acids, to copherols *inter alia* (Ali *et al.*, 2010). Dixon (1999) while studying the biochemistry of flavonoids stated that they play a vital role of resistance in legumes. This, they achieve via their capacity to modulate the feeding behavior of insects (Hedin and Waage, 1986). Tannins have been reported to exert their action on plant pests by a combination of mechanisms that includes iron chelation and enzyme inhibition (Karamanoli *et al.*, 2011).

Further still, Dolui *et al.* (2010) reported that tannins combined with protein to inhibit enzyme activity and reduce the availability of protein in haemolymph in insects. Chaieb (2010) extensively reviewed insecticidal effects of saponins, linking their insecticidal activity with cholesterol which results in impaired ecdysteroid synthesis. Some of the reported observed effects of saponins are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction (*ibid.*). Plant alkaloid toxicity can be quite diversified, but often involves neurotoxicity or cell signaling disruption according to Mithöfer and Boland (2012). The authors noted further that toxicity also arose by enzymatic alterations that affect physiological processes, inhibits synthesis of DNA and repair mechanisms by intercalating with nucleic acids. The negative effects of their contact with pests include reduced ovary development, mobility, and survivorship (*ibid.*).

In fact, complex mixtures of secondary compounds in plant extracts were reported to contribute to a great deal for synergism, which enhances the joint action of active compounds against insect and reduces the rate of resistance development (Feng and Isman, 1995).

Table.1 Percentage yield of aqueous extract of the pesticidal pests

Crude extract	% Yield
<i>A. indica</i>	4.78
<i>C. frutescens</i>	11.69
<i>T. minuta</i>	4.96
<i>Allium cepa</i>	9.84
<i>P. dodecandra</i>	4.75

Table.2 Phytochemical components of the pesticidal plants extracts

Plant sample	Alkaloids	Tannins	Steroids	Flavonoids	Saponins
<i>P. dodecandra</i>	+	+++	–	+++	+++
<i>Allium cepa</i>	+	+	+	+++	–
<i>C. frutescens</i>	+	+	+	+++	+
<i>A. indica</i>	+	++	+	+++	–
<i>T. minuta</i>	+	++	+	–	–

KEY-Heavily present: +++ Slightly present: ++ Present: + Absent: –

Table.3 Quantitative estimates of phytochemicals of extracts from sample plants

Sample	Alkaloids (mgml ⁻¹)	Tannins (mgml ⁻¹)	Steroids (mgml ⁻¹)	Flavonoids (%)	Saponins (%)
<i>P. dodecandra</i>	0.1312 ± 0.000 ^a	0.1537 ± 0.008 ^a	–	12.134 ± 0.006 ^a	31.059 ± 0.062 ^a
<i>Allium cepa</i>	0.0871 ± 0.001 ^b	0.0407 ± 0.002 ^b	0.5248 ± 0.017 ^a	20.534 ± 0.064 ^b	–
<i>C. frutescens</i>	0.1045 ± 0.000 ^c	0.34 ± 0.014 ^c	2.2791 ^b	26.134 ± 0.058 ^c	7.786 ± 0.058 ^b
<i>A. indica</i>	0.0889 ± 0.001 ^b	0.3346 ± 0.020 ^b	2.2791 ^b	21.266 ± 0.064 ^c	–
<i>T. minuta</i>	0.1560 ± 0.000 ^d	0.4494 ± 0.027 ^d	0.9387 ± 0.013 ^c	–	–
p-value	0.000	0.000	0.000	0.000	0.000

Values are means ± standard deviation of three replicates (N=3), means in a column with the same letter are not significantly different (p > 0.05).

Fig.1 Standard calibration curve of quinine sulphate stock solution

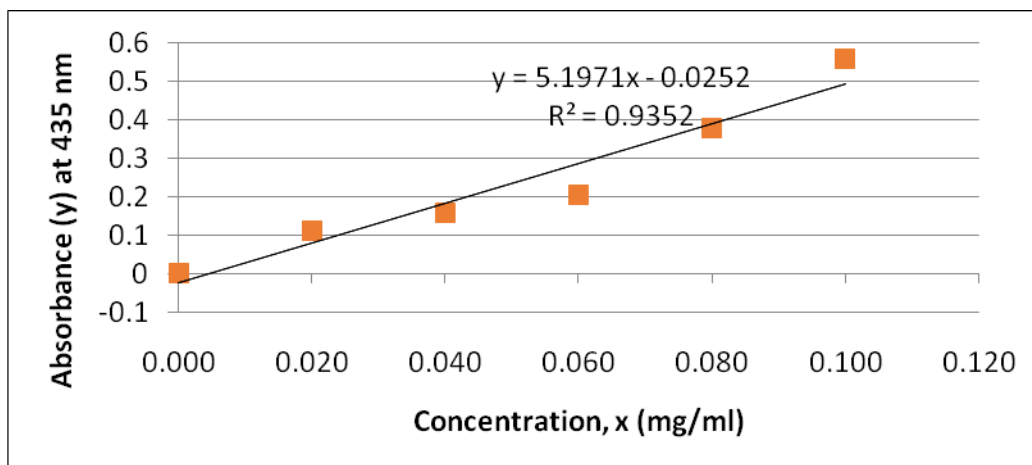


Fig.2 Standard calibration curve of tannic acid stock solution

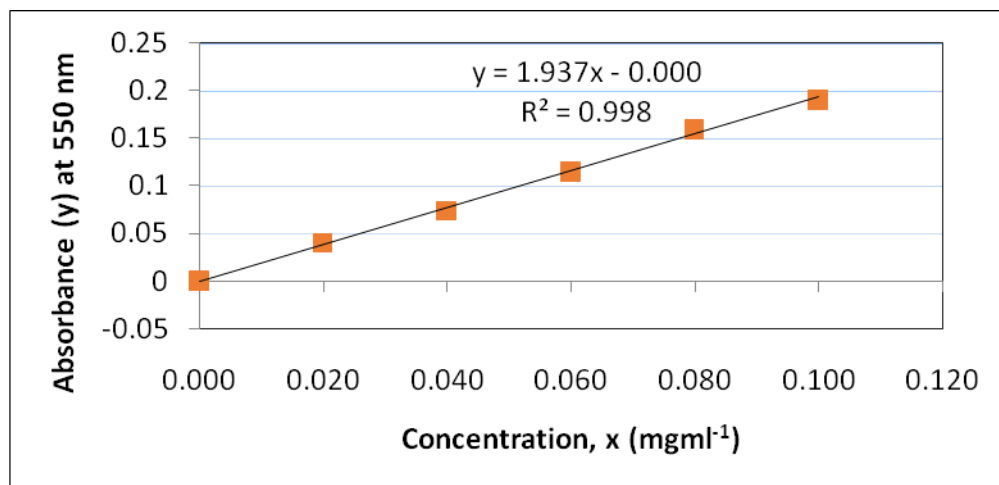
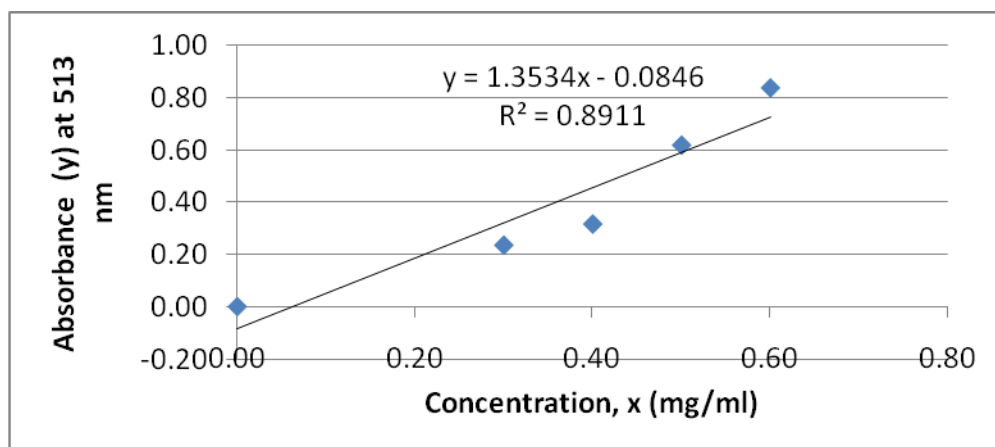


Fig.3 Standard calibration curve of steroid standard stock solution



Notwithstanding, Anupam *et al.*,(2012)noted that the effectiveness of phytochemicals against crop pests can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), as well as the solvent used during extraction. Trease and Evans (2009)go ahead to state that the quantity and the composition of bioactive compounds present in plants are influenced by the genotype, extraction procedure, geographic and climatic conditions, and the growth phase of the plants. They continue to say that this makes it difficult to compare data with the literature because several variables influence the results.

Results from the present investigation showed that *A. indica*, *T. minuta*, *P. dodecandra*, *Allium cepa* and *C. frutescens* are very rich in phytochemicals, even though the phytochemical analysis of the plants revealed some differences in their constituents. However, aqueous extracts of *C. frutescens*, *A. indica* and *T.minuta* had higher quantities of bioactive compounds than *Allium cepa*, and *P. dodecandra* extracts contributing to their increased effectiveness for pest control.

These results suggest that these plants are a valuable reservoir of bioactive compounds of substantial pesticidal merit. Aqueous extracts

of *C. frutescens*, *A. indica* and *T.minuta* are especially beneficial for managing Brassica vegetable pests as they contained the highest quantities of the bioactive components. This study may be useful in exploring the pharmacological and biosynthetic activity of these plants further.

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