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RESEARCH PAPER

# Yield, yield features, phytochemical composition, antioxidant and antibacterial activities of *Abutilon indicum* cultivated under different fertilizers

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**Abstract**

**G. Yaldiz, A. B. Yildirim, Y. K. Arici, and M. Camlica. 2016. Yield, yield features, phytochemical composition, antioxidant and antibacterial activities of Abutilon indicum cultivated under different fertilizers. Cien. Inv. Agr. 43(3):464-475.** *Abutilon indicum* (Link) Sweet is an important medicinal plant that has been used in traditional medicine for centuries, and it is one of the novel crops being used in the pharmaceutical industry. Agricultural practices that produce high drug yield and desired secondary metabolites are in high demand by pharmaceutical-related industries; therefore, the aim of this study was to determine the leaf and seed yield, crude oil yield, crude oil composition, total phenolic content, and the antioxidant and antibacterial potential of *A. indicum* Sweet grown using two fertilizer applications: a three-component fertilizer containing nitrogen, phosphorus and potassium (NPK, 10-10-40) and calcium ammonium nitrate (CAN, 26% N). In addition, the results were compared to a control group in which no fertilizer was used. Two experiments were conducted in two consecutive years, and the highest leaf and seed yields, 74250.0±5440.0 kg $ha^{-1}$ and 1159.6±62.2 kg $ha^{-1}$, respectively, were obtained from the CAN application in both years. Seed crude oil content varied from 13.6 to 14.7%, and linoleic acid (69.5%) was the major crude oil acid in the seed oil, which reached higher values under the NPK than the CAN application. Both fertilizers were found to promote the highest antioxidant activity in the roots (87.2%). In leaves, the highest phenolic compound content was observed in the control application (56.9±0.0 mg GAE $g^{-1}$ dry extract), and the highest flavonoid content was detected in the CAN application (107.4 mg Pyrocatechol $g^{-1}$ dry extract). Except the leaves extracts, the root and seed extracts of *A. indicum* Sweet had higher phenolic contents than the control application. For all of the analyzed extracts combined (leaf, root and seed), the fertilized plants had higher flavonoid contents than the control application. In addition, the root extracts of *A. indicum* Sweet exhibited the highest antibacterial activity against *Streptococcus pyogenes.*

**Keywords:** Biological activity, CAN, crude oil, fertilizer, NPK, yield.

## Introduction

Increasing risks of infection stemming from antibiotic-resistant microorganisms have made the discovery of new and natural antimicrobial substances the focus of various studies, while the expectations of conscious consumers have encouraged or even forced drug producers and service providers to use natural preservatives. Thus, a need has arisen to investigate and test the efficacy of various plants against microorganisms, and the

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antibacterial impacts of various plant extracts on microorganisms and, particularly, food pathogens have been reported by several researchers (Prescott *et al*., 1990). It is well known that reactive oxygen compounds such as singlet oxygen, superoxide radicals, hydrogen peroxide, hydroxyl radicals and nitric oxide are unstable and extremely reactive, and oxidative stress-induced reactive oxygen species are considered indicators of the development and progress of various cardiovascular diseases. Antioxidants prevent the negative impacts of free radicals and reactive oxygen species and protect the body. Today, the most common synthetic antioxidants are butylated hydroxyltoluene (BHT), butylated hydroxylanisole (BHA), propyl gallate and tertiary butylhydroquinone (Fisherman and Cohen, 1973).

However, the use of synthetic antioxidants has been debated due to their toxic and carcinogenic effects, which can result in liver damage, so the discovery of new, reliable and harmless antioxidants from natural resources has become a prominent research topic (Birman, 2012). *Abutilon indicum* Sweet is an herbaceous and shrubby plant in the *Malvaceae* family that grows in tropical and subtropical regions, and it contains saponins, flavonoids, alkaloids and essential oil components such as $\beta$-sitosterol, $\beta$-amyrin, eudesmol, eugenol, geraniol and caryophyllene (Yasmin *et al*., 2008). Therefore, it has been used as a diuretic, pulmonaric, sedative, laxative, antilipidemic and an emollient in folk medicine (Tripathi *et al.,* 2012). The whole plant has also been used to cure blood dysentery and treat allergies, and the seeds are an efficient laxative and expectorant for cough treatment (Yasmin *et al*., 2008). The leaves have been used to treat throat ulcers, headaches, gonorrhea, and bladder infections and have been employed in the traditional phytotherapy of jaundice and hepatoprotective diseases (Porchezhian and Ansari, 2005). *A. indicum* roots are demulcents and diuretics that are prescribed for fever, chest infection and urethritis (Ponnudurai *et al*., 2011), and the plant has also been reported to have hepatoprotective (Porchezhian and Ansari, 2005; Tripathi *et al.,* 2012), analgesic, antifertility, antifungal, antibacterial (Tripathi *et al*., 2012), immunological and anti-inflammatory (Yasmin *et al.,* 2008) activities.

Fertilization is one of the most significant agricultural practices used to improve the yield and quality of traditional crops. Nitrogen is an essential element for plant development that is present in several chemical forms in nature and in chemical compounds such as ammonium, nitrate and urea. Chemical nitrogen fertilizer mixtures such as a three-component fertilizer containing nitrogen, phosphorus and potassium (NPK, 10-10-40) and calcium ammonium nitrate (CAN, 26% N) are examples of combining these compounds. The availability of nitrogen in plants varies with the different forms and mixtures of nitrogen (Mattson and Leatherwood, 2009). In this study, the adaptability of *A. indicum* Sweet to the climatic conditions of the Eastern Black Sea region of Turkey was investigated by determining the leaf and seed yield, crude oil yield, crude oil composition, total phenolic content, and the antioxidant and antibacterial potential of this plant under different fertilizer applications.

## Materials and methods

### *Growth conditions*

Field experiments were carried out during two successive seasons (2012 and 2013) at Ordu University Experimental Farms (40°58’36’’ N, 37°59’55’’ E), located at an altitude of approximately 10 m above sea level. Climatic data were recorded for both years during the summer growing seasons (from April to August), and the means were as follows: a temperature of 19 °C, 270 mm of precipitation and 69% relative humidity. The soil in the experimental area was clay-loam with a pH value of 7.8, organic matter content of 4.7%, phosphorus content of 10.3 ppm and potassium ratio of 235 ppm. The experiment was implemented in a randomized block design with three

replications, and each experimental plot consisted of five 14-m long rows with an inter-row distance of 0.5 m and an inter-plant distance of 0.3 m; the total number of plants in each plot was 75. As the base fertilizer, NPK (25-15-0) was added at the rate of 60 kg $ha^{-1}$ during soil preparation, and the different experimental fertilizer applications, 100 kg $ha^{-1}$ CAN (26% N) and 100 kg $ha^{-1}$ NPK (10-10-40), were the applied to the plots. In both years, the plants ripened after 90-100 days following the spring sowing and were then harvested twice a week until the end of the season. Before the harvest, the yield components, namely, plant height, the number of branches and the number of capsules in the main branches were measured, and laboratory analyses were then performed on the seeds, leaves and roots.

*Isolation of crude oil*

Seed crude oil yield was calculated on a 91% dry matter basis, and the oil content was determined using the Soxhlet method, in which seed samples were finely ground in a coffee grinder (manufactured by Bran, Punjab Engineering Works, Mohali, Punjab, India) and extracted with *n*-hexane in a Soxhlet apparatus over 8 h at a constant temperature of 80 °C (James, 1995).

*GC-MS/FID analysis of crude oil composition*

Seed crude oil compositions were analyzed using gas chromatography (Agilent 7890A) coupled with a flame ionization detector and mass spectrometry (Agilent 5975C) with a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm; 0.25 µm film thickness). The compositions were diluted with hexane at a ratio of 1:50, and GC-MS/FID analyses were performed at the split mode of 50:1. The injection volume and temperature were adjusted to 1 mL and 250 °C, respectively, and helium (99.9%) was used as the carrier gas at a constant flow rate of 1 mL $min^{-1}$. The oven temperature was programmed to increase at a rate of 20 °C/min from 60 °C for 10 min to 250 °C and then remain at this temperature for 8 min. MS spectra were monitored within the range of 35-450 amu, and electronic impact at 70 eV was used as the ionization mode. The relative percentage of the components was calculated from GC-FID peak areas, and the components were identified using the WILEY, NIST and FLAVOR libraries.

*Antibacterial properties of Abutilon*

Twenty-five g of each harvested plant part were extracted with 300 mL methanol over 18 h in a water bath at 40 °C and then filtered. Filtrates were evaporated under a vacuum using a rotary evaporator and then dissolved in 10 mL of distilled water and lyophilized. All of the extracts were stored at -20 °C until use (Table 1).

A disc diffusion assay (Kirby–Bauer Method) was performed to screen for antibiotic activity (Prescott and Harley, 1990), and the following gram positive bacterial strains were used: *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. BD-Microtrol discs (Becton Dickinson Laboratories, France) containing different bacterial strains were transferred to test tubes containing 2 mL of tryptic soy broth (TSB) and incubated for 3 h at 37 °C. After 3 h, one bacteriological loop from each broth was streaked on tryptic soy agar (TSA) plates and incubated for 2 days at 37 °C, after which a single colony was removed and streaked on a new TSA plate and incubated at 37 °C for 2 additional days. Then, 4-5 loops of pure culture were transferred to 20 mL of TSB in a test tube for each bacterial strain and incubated overnight at 37 °C. Each bacteria broth culture was adjusted with saline to obtain a turbidity that was visually comparable to a 0.5 McFarland standard, and Mueller Hinton agar plates for each bacterium were then inoculated using cotton swabs. All extracts were sterilized by filtering through a 0.22-mm filter (Pal-Gelman Laboratory), and sterile filter paper discs (Glass Microfiber filters, Whatman®; 7 mm in diameter) were impregnated with 13

**Table 1.** Comparison of the agronomic properties and yield of *A. indicum* grown under different fertilizer applications.

| | Year | Control | NPK | CAN | Average | P-value |
|---|---|---|---|---|---|---|
| Plant height (cm) | 2012 | 137.7±3.8Ba | 178.1±19.1ABa | 216.4±17.0Aa | 177.4±13.6 | Y:0.000 NS |
| | 2013 | 110.4±9.0Aa | 139.0±14.6Aa | 122.1±10.80Ab | 123.8±7.12 | T:0.010 NS |
| | Avr. | 124.1±7.5 | 158.5±13.9 | 169.2±22.9 | | Y*T:0.038[1] |
| Number of branches | 2012 | 6.2±0.3 | 5.2±0.1 | 6.9±1.2 | 6.1±0.4 a | Y:0.013[1] |
| | 2013 | 4.6±0.2 | 4.8±0.5 | 4.5±0.3 | 4.7±0.2 b | T:0.501 NS |
| | Avr. | 5.4±0.4 | 5.0±0.2 | 5.7±0.8 | | Y*T:0.265 NS |
| Number of seeds per capsule | 2012 | 38.4±2.2 | 40. 0±1.1 | 40.4±0.7 | 39.6±0.8 a | Y:0.001[2] |
| | 2013 | 31.4±1.1 | 27.5±0.5 | 32.0±3.7 | 30.3±1.3 b | T:0.526 NS |
| | Avr. | 34.9±1.9 | 33.8±2.9 | 36.3 ±2.5 | | Y*T:0.446 NS |
| Seed weight per plant (g) | 2012 | 13.4±0.8 | 14.4±0.7 | 14.5±1.2 | 14.1±0.45 a | Y:0.004[2] |
| | 2013 | 9.4±0.7 | 10.7±0.6 | 7.8±3.3 | 9.3±1.1 b | T:0.633 NS |
| | Avr. | 11.4±1.0 | 12.5±0.9 | 11.2±2.1 | | Y*T:0.552 NS |
| 1000-seed weight (g) | 2012 | 11.1±0.1 | 10.7±0.2 | 11.7±0.1 | 11.2±0.2 a | Y:0.000[3] |
| | 2013 | 8.4±0.4 | 8.1±0.6 | 9.5±0.3 | 8.7±0.3 b | T:0.034[1] |
| | Avr. | 9.7±0.6 AB | 9.4±0.7 B | 10.6±0.5 A | | Y*T:0.781 NS |
| Capsule breadth (cm) | 2012 | 0.6±0.0 | 0.7±0.0 | 0.7±0.0 | 0.67±0.0 a | Y:0.000[2] |
| | 2013 | 0.5±0.1 | 0.6±0.0 | 0.6±0.0 | 0.56±0.0 b | T:0.357 NS |
| | Avr. | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 | | Y*T:0.661 NS |
| Capsule length (cm) | 2012 | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 | 0.56±0.0 a | Y:0.007[2] |
| | 2013 | 0.5±0.0 | 0.5±0.0 | 0.4±0.0 | 0.5±0.0 b | T: 0.789 NS |
| | Avr. | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 | | Y*T:0.996 NS |
| Fresh leaf yield (kg ha$^{-1}$) | 2012 | 62050.0±2720.0 | 64643.0±852.0 | 74250.0±5440.0 | 66980.0±2570.0 a | Y:0.002[2] |
| | 2013 | 51870.0±1870.0 | 53143.0±996.0 | 63680.0±3190.0 | 56230.0±2170.0 b | T:0.006[2] |
| | Avr. | 56960.0±2710.0 B | 58890.0±2640.0B | 68970.0±3680.0 A | | Y*T:0.971 NS |
| Dry leaf yield (kg/ha) | 2012 | 15730.0±1140.0 | 15416.0±366.0 | 19410.0±2000.0 | 16850.0±929.0 a | Y:0.026[1] |
| | 2013 | 14060.0±1480.0 | 12730.0±102.0 | 17070.0±1010.0 | 14619.0±875.0 b | T:0.007[2] |
| | Avr. | 14893.0±916.0 B | 14075.0±772.0 B | 18240.0±1130.0 A | | Y*T:0.879 NS |
| Seed yield (kg/ha) | 2012 | 794.0±40.2 | 1023.3±18.2 | 1159.6±62.2 | 992.2±57.7a | Y:0.000[3] |
| | 2013 | 667.0±25.0 | 716.3±71.4 | 760.3±91.9 | 714.6±36.9 b | T:0.006[2] |
| | Avr. | 731.0±35.4 B | 869.8±76.1 AB | 960.0±102.0 A | | Y*T:0.095 NS |

Y, year; T, treatment; Y*T, year*treatment interaction; mean±SEM; NS: not statistically significant (P>0.05).
[1]Statistically significant (P≤0.05); [2]statistically significant (P≤0.01); [3]statistically significant (P≤0.001).
Horizontally, means that do not share a capital letter are significantly different from each other (P≤0.05).
Vertically, means that do not share a lowercase letter are significantly different from each other (P≤0.05).

µL of extract. There were five replicates on each plate, and two plates for each extract were tested for each bacterium. Positive controls consisted of two different antimicrobial susceptibility test discs (Bioanalyse®): ampicillin (10 mg) (AM-10) and tetracycline (30 mg) (TE-30). Two antibiotic discs were used for each plate and run in duplicate, and the negative control was water. Inoculated plates with discs were placed in a 37 °C incubator. After 16 to 18 h of incubation, the diameter (mm) of the inhibition zone was measured. All experiments were repeated three times.

*Antioxidant properties of Abutilon*

The free radical scavenging activity of the methanolic extracts of *A. indicum* Sweet leaves, roots and seeds was determined spectrophotometrically by monitoring the disappearance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 517 nm, according to the method described by Brand-Williams *et al.* (1995). Briefly, a 0.15-mM solution of DPPH was prepared in methanol, and 1 mL of this solution was then added to 3 mL of the extracts at different concentrations (25, 50, 100 and 200 µg $mL^{-1}$). These solutions were incubated in the dark, and the absorbance of each was measured at 517 nm against blank samples using a 200-V Hitachi U-1900 UV-VIS spectrophotometer. All analyses were performed in triplicate, and the DPPH scavenging capacity of the extracts was calculated using the following equation (Gulcin *et al*., 2003):

$$\text{DPPH·Scavenging Effect (\% inhibition)} = [(A_0 - A_1 / A_0) \times 100] \quad (1)$$

$A_0$: Absorbance of the control reaction

$A_1$: Absorbance in the presence of the tested extracts

*Phenolic content of Abutilon*

The total phenolic content in the methanolic extracts of *A. indicum* Sweet leaves, roots and seeds was determined by the procedure described by Slinkard and Singleton (1977) with the slight modification of using a Folin-Ciocalteu phenolic reagent. Gallic acid was used as a standard phenolic compound. Briefly, 2 mL of distilled water were added to 0.01 g of the *A. indicum* extracts (5 mg $mL^{-1}$), and the prepared stock solution was then diluted to 1 mg $mL^{-1}$. To prepare a calibration curve, solutions of 0, 25, 50, 100, 150 and 200 mg $L^{-1}$ gallic acid were prepared, and 20 µL from each calibration solution, sample, or blank were placed into separate cuvettes. Then, 1.58 mL of water and 100 µL of Folin-Ciocalteu reagent (Sigma®) were added to each cuvette and mixed well. After 2 minutes, 300 µL of $Na_2CO_3$ solution were added to the mix and thoroughly shaken. These solutions were incubated at 20 °C for 2 h, and the absorbance of each solution was measured at 765 nm against the blank using a spectrophotometer. The amounts of total phenolic compounds in *A. indicum* leaf, root and seed extracts were determined as micrograms of gallic acid equivalent (GAE) using an equation obtained from a standard gallic acid graph ($R^2$: 0.9957). All of the analyses were performed in triplicate.

*Flavonoid content of Abutilon*

The total flavonoid content in the methanolic extracts of *A. indicum* leaves, roots and seeds was measured by aluminum chloride ($AlCl_3$) colorimetric assay. Catechol was used as a reference flavonoid, and 2500 and 1250 mg $mL^{-1}$ concentrations of the extracts were prepared in ethanol. Different catechol concentrations (20, 40, 60, 80 and 100 mg $mL^{-1}$) were prepared to obtain a standard catechol calibration curve. Briefly, 500 µL of extract solution or standard catechol solution was added to a 10-mL test tube containing 2 mL of distilled water, and 150 µL of 5% $NaNO_2$ was then added to each test tube. After 5 min, 150 µL of 10% $AlCl_3$ was added, and at 6 min, 1000 µL of 1 M NaOH was added to the mixture followed by dilution to a volume of 5 mL by adding 1200 µL of distilled water and mixing thoroughly. The

absorbance of the mixture was determined at 510 nm against a blank. The samples were analyzed in three replicates (Marinova *et al*., 2005), and the total flavonoid contents of *A. indicum* leaf, root and seed extracts were given as mg catechol equivalents (CE) per 100 $g^{-1}$ extract dry weight.

*Statistical analysis*

Anderson-Darling and Bartlett tests were applied to test normality and homogeneity of variance, respectively. Agronomic parameters were analyzed by two-way ANOVA (with repeated block experiments in different years), and the seed crude oils were analyzed by one-way ANOVA. The means were compared with the Tukey HSD test, and the results are displayed as letters associated with the mean. All parameter values are expressed as the mean±standard error of the mean (SEM)/standard deviation (SD), and the alpha level was set at 5%. The statistical analysis was performed using the Minitab v17 (Minitab Inc., State

College, Pennsylvania, USA). The results of the antibacterial bioassays were statistically analyzed using ANOVA and Duncan's multiple range test.

**Results**

*Plant development*

Based on the data obtained from the field trial, there were no significant differences in the plant height of *A. indicum* between fertilizer applications. The highest plant height value was obtained from the CAN application in the first year, while in the second year, it was obtained from NPK. The number of branches varied from 4.5 to 6.9 branches $plant^{-1}$, with the highest average number obtained in the CAN fertilizer treatment. The number of seeds per capsule ranged from 27.5 to 40.4, with the highest value observed with CAN and the lowest with NPK. There were no significant differences between fertilizer applications in terms of the seed weight, but the highest thousand-seed weight was obtained from the CAN fertilizer application, while the lowest was observed in the NPK application. The thousand-seed weight was different between the first and second experimental years with higher values in the first year.

The average capsule width varied between 0.5 and 0.7 cm, and the highest value was obtained from both fertilizer applications. In both experimental years, the length of the capsule grown under different fertilizer applications ranged from 0.4 to 0.6 cm, with the longest capsule obtained from the CAN application in the first year, and the shortest capsule was obtained from the control in the second year. The *A. indicum* fresh leaf yield was significantly different between the two years, ranging from 51.870 to 74.250 kg $ha^{-1}$ with the different applications. The highest leaf yield was obtained from the CAN application in both years, and the lowest total fresh herbal weight was from the control application: 62.050 kg $ha^{-1}$ in the first year and 51.870 kg $ha^{-1}$ in the second year. The highest dry leaf yield was obtained from the CAN application: 19.410 and 17.070 kg $ha^{-1}$ in the first and second years, respectively. The lowest dry leaf yield was obtained from the NPK treatment in both years, 15.410 and 12.730 kg $ha^{-1}$, respectively.

Although there were significant differences between years in terms of seed yields, no significant differences were observed between the fertilizer applications. In the present study, the seed yield of *A. indicum* ranged widely from 667 to 1159.6 kg $ha^{-1}$ with the highest seed yield obtained from the CAN application (Table 1).

*Chemical properties of crude oil*

Table 2 presents the chemical properties of *A. indicum* crude oil by fertilizer treatment, and there were significant differences in seed crude oil content between the fertilizer applications.

The crude oil content ranged from 13.6 to 14.7%, with the highest value observed with the NPK application. Different fertilizer applications had a statistically significant effect on seed crude oil acids, except for the palmitic and linolenic acids. Among the fertilizer applications that statistically affected the crude oil acid contents, NPK yielded the highest values (Table 2).

*Antibacterial properties*

Nine different plant extracts (leaf, root and seed MeOH extract of *A. indicum* grown in CAN- and NPK-treated and control soils) were screened for antibacterial activity. Bacterial growth was generally sensitive to the tested reference antibiotics, and no inhibition was observed with the extraction solvent (water). All methanol extracts only exhibited antibacterial activity against *S. pyogenes*, and when compared to chloramphenicol (reference antibiotic) (37.0 ± 1.7 mm), the extracts showed lower antibacterial activity. Among the leaf, root and seed methanol extracts, the root extracts had the highest antibacterial activity against *S. pyogenes*, and the CAN and control extracts exhibited higher activity in the root (11.5- and 11.3-mm inhibition zone, respectively) than the NPK extract (9.5-mm inhibition zone). The tested methanol extracts displayed no antibacterial activity against *S. aureus* or *S. epidermidis*.

*Antioxidant properties and secondary metabolite contents*

The antioxidant activity of the methanolic extracts of *A. indicum* leaves, roots and seeds grown in soils treated with CAN or NPK and the control were examined using the DPPH scavenging method. The results indicated that at a minimum concentration (25 µg $mL^{-1}$), all of the extracts had less than 50% DPPH radical scavenging activity, but when used at a concentration of 50 µg $mL^{-1}$, the control leaf and NPK root extracts displayed higher radical scavenging activities (60.6 and 52.4%, respectively) than the other extracts.

At 100 µg $mL^{-1}$ and above, all of the leaves extracts, except CAN leaf extracts, exhibited scavenging activities greater than 50% DPPH (Table 3); in other words, the extracts obtained from *A. indicum* seed treated with CAN, NPK or grown on control soils had little tendency to scavenge DPPH radicals compared to the other extracts. When leaves grown on CAN- and NPK-treated and control soils were compared, the control leaf extract showed the highest DPPH radical scaveng-

**Table 2.** *Comparison of the* crude oil yield and composition of *A. indicum* seeds (%) grown under different fertilizer applications.

| | Soil treatment | | | | |
|---|---|---|---|---|---|
| Variables | RT | Control | NPK | CAN | P-value |
| Seed crude oil content | - | 13.6±0.1B | 14.7±0.1A | 13.6±0.3B | 0.004[1] |
| Palmitic acid (C16:0) | 16.4 | 15.6±0.0 | 15.9±0.1 | 15.5±0.2 | 0.465 NS |
| Palmitoleic acid (C16:1) | 16.9 | 0.3±0.0 B | 0.4±0.0A | 0.4±0.0B | 0.005[1] |
| Stearic acid (C18:0) | 20.2 | 3.0±0.0 | 3.0±0.0 | 2.9±0.0 | 0.276 NS |
| Oleic acid (C18:1) | 20.7 | 11.1±0.0 B | 11.9±0.0A | 10.9±0.1C | 0.000[2] |
| Linoleic acid (C18:2) | 21.7 | 69.2±0.0 A | 69.5±0.1A | 67.9±0.1B | 0.000[2] |
| Linolenic acid (C18:3) | 23.0 | 0.8±0.0 | 0.8±0.0 | 0. 8±0.0 | 0.264 NS |

NS: Not statistically significant (P>0.05).
[1] Statistically significant (P≤0.01); [2] statistically significant (P≤0.001).
Means that do not share a letter are significantly different from each other (P≤0.05).
RT: Retention time.

ing activity at all of the studied concentrations. Among the fertilizer applications, leaves treated with NPK showed higher DPPH scavenging activity than leaves treated with CAN. When roots treated with CAN and NPK and the control were compared, the CAN and NPK root extracts showed higher DPPH scavenging activities than the control root extract.

Table 4 presents the total phenolic and flavonoid contents of the leaf, root and seed extracts grown on soil treated with CAN and NPK and the control. The control leaf extract possessed the highest concentration of phenolic compounds (56.9±0.0 mg GAE $g^{-1}$ dry extract) compared to the other extracts followed by the leaf and root extracts grown on the NPK soil (46.4 and 46.1 mg GAE $g^{-1}$ dry extract, respectively). The seed extracts grown on CAN and NPK soil had higher phenolic compound contents (15.1 and 20.5 mg GAE $g^{-1}$ dry extract, respectively) than the control seed extract, and similarly, when the root extracts were compared, the CAN and NPK treatments yielded higher phenolic contents (41.8 and 46.1 mg GAE $g^{-1}$ dry extract, respectively) than the control root extract. However, among the leaf extracts, the control leaf extract had a higher phenolic content (56.9 mg GAE $g^{-1}$ dry extract) than the CAN and NPK leaf extracts (Table 4).

The CAN leaf extract was found to possess the highest flavonoid content (107.4 and 84.6 mg pyrocatechol $g^{-1}$ dry extract), and the CAN and NPK leaf, root and seed extracts had higher flavonoid contents than all of the control extracts. In addition, the CAN leaf and seed extracts contained a higher flavonoid percentage compared to the NPK extracts. In contrast, the NPK root extract had a higher flavonoid content than the CAN root extract (Table 4).

## Discussion

When the results from this study are compared to those of previous reports, there are some similarities and differences. In the present study, plant height ranged from 110.4 to 216.4 cm with the different fertilizer applications, while this range is between 100 and 200 cm in other studies (Sharma *et al*., 2013). Parrish and Bazzaz (1985) reported the number of seeds in each capsule to be 16.5-31.5, with the nutritional value increasing as the number of capsules increased, but in the present study, the capsule number was found to be much higher. These differences are not surprising since plant properties are highly dependent on environmental conditions and the agricultural techniques being applied. In the present study, the highest agronomical and yield properties were obtained from the CAN treatment followed by the NPK and control applications (Table 1). There are many scientific reports showing that fertilizers containing a higher level of nitrogen improve the yield and yield components (Zareie *et al*., 2011); similarly, the CAN fertilizer that had the highest nitrogen content (26% N) produced the highest average yield value in the present study (Table 1). However, the highest crude oil content and most desired crude oil composition were obtained from the NPK fertilizer treatment. Some studies have reported that potassium has a positive effect on the secondary metabolite content in plants (Hu and Schmidhalter, 2005), and we obtained higher values of secondary metabolites with K fertilization (Table 2). The major components of *A. indicum* seed oil were identified as linoleic, oleic, stearic and palmitic acids, and this result is consistent with earlier reports (Sharma *et al.,* 2013). These oil components promoted antioxidant activity, as reported in previous studies (Yasmin *et al*., 2008; Tripathi *et al.,* 2012), and oleic acid may also play an important role in the treatment of heart disease (Singh and Gupta, 2008).

In this study, all of the methanol extracts only exhibited antibacterial activity against *S. pyogenes*, and the highest antibacterial activity was observed by the root extract from the CAN fertilizer treatment against *S. pyogenes*. Yasmin *et al*. (2008) reported that the root parts of *A. indicum* exhibited greater antibacterial activity against *Micrococcus luteus, Bacillus subtilis, Escherichia*

**Table 3.** Free-radical (DPPH●) scavenging activity (%) of the methanolic extracts of *A. indicum* leaves, seeds and roots and of ascorbic acid (positive control) grown under different fertilizer applications.

| | Concentration (µg mL$^{-1}$) | | | |
|---|---|---|---|---|
| Treatments | 25 | 50 | 100 | 200 |
| Ascorbic acid | 95.8 | 96.8 | 97.6 | 97.3 |
| CAN Leaf | 5.7 | 20.7 | 21.4 | 42.7 |
| NPK Leaf | 14.8 | 38.5 | 73.3 | 80.7 |
| Control Leaf | 20.5 | 60.6 | 78.2 | 86.7 |
| CAN Seed | 10.6 | 14.9 | 18.7 | 31.0 |
| NPK Seed | 13.7 | 13.7 | 13.4 | 27.7 |
| Control Seed | 10.0 | 13.7 | 18.5 | 19.3 |
| CAN Root | 20.8 | 40.0 | 60.3 | 87.2 |
| NPK Root | 43.1 | 52.4 | 81.1 | 87.2 |
| Control Root | 20.0 | 36.3 | 54.1 | 75.8 |

**Table 4.** Total phenolic and flavonoid contents of the methanolic extracts of *A. indicum* leaves, seeds and roots grown under different fertilizer applications.

| | Leaf | Seed | Root | Leaf | Seed | Root |
|---|---|---|---|---|---|---|
| Soil treatment | Total phenolics per mg GA g$^{-1}$ dry extract | | | Total flavonoids per mg pyrocatechol g$^{-1}$ dry extract | | |
| CAN | 34.7 ± 0.0 | 15.1 ± 0.0 | 41.8 ± 0.0 | 107.4 ± 0.0 | 76.1 ± 0.0 | 47.7 ± 0.0 |
| NPK | 46.4 ± 0.0 | 20.5 ± 0.0 | 46.1 ± 0.0 | 84.6 ± 0.0 | 26.2± 0.0 | 86.8 ± 0.0 |
| Control | 56.9 ± 0.0 | 14.5 ± 0.0 | 33.5 ± 0.0 | 61.7 ± 0.0 | 13.2 ± 0.0 | 24.8 ± 0.0 |

Mean±SD.

*coli* and *Bacillus licheniformis* compared to the leaf and seed extracts, which is consistent with the results of the present study.

Similar to the present findings, Raman *et al.* (2009) reported that methanol, ethanol and mixed (methanol: chloroform: water) *A. indicum* extracts showed no antibacterial activity against *S. aureus* and the tested microorganisms. Abdul *et al.* (2010) also indicated that n-hexane and methanol fraction extracts were not active against *S. aureus,* and the chloroform fraction extract only exhibited antimicrobial activity against *Sarcina lutea* (8.4 mm). In contrast to our findings, Gurumurthy *et al.* (2011) reported that the methanolic extract of *A. indicum* had potent antibacterial effects on *S. aureus*, and Ranjit *et al.* (2013) indicated that the chloroform extract of *A. indicum* leaves showed antimicrobial activity against gram (+) microorganisms (19.3±0.5 mm in diameter against *S. aureus*). In our study, each bacterium was inoculated on Muller Hinton Agar plates, and filter paper discs were impregnated with 13 mL of methanol extract, whereas the above-mentioned researchers used nutrient agar media for the microorganism inoculum and impregnated discs with higher volumes of the extracts (20 or 100 µL). It is obvious that differences in the extraction methods, extract concentrations and bacteriological media affected the biological activity results. The leaves of *A. indicum* exhibited good antioxidant activity in the NPK and control applications while the highest antioxidant activity among the *A. indicum* root extracts was observed in the NPK and CAN plots at a concentration of 200 µg mL$^{-1}$ (Table 3). The total phenolic and flavonoid contents of *A. indicum* varied with the fertilizer application, with the highest value found in both the NPK and CAN treatments, and the highest

phenolic and flavonoid contents were observed in the leaf extracts (Table 4). Thus, based on the content of secondary metabolites, the fertilized plants had higher biological activity. Similarly, Sharma *et al*. (2013) evaluated the free radical scavenging activity based on the nitric oxide and superoxide radical scavenging activities and found the maximum values to be 28.74% and 49.62%, respectively, at a concentration of 250 µg $mL^{-1}$.

The total phenolic and flavonoid contents of *A. indicum* have also been investigated by other researchers to assess antioxidant capacity. Chakraborthy and Ghorpade (2010) reported that the total phenolic contents of *A. indicum* stem extracts (methanolic, hydro-alcoholic and aqueous), as measured by Folin Ciocalteu reagents in terms of a gallic acid equivalent (GAE), were 20.94, 27.77 and 35.45 mg $g^{-1}$, respectively. Furthermore, with DPPH at IC50 (the concentration that inhibits 50% of the DPPH radical), the authors reported that the methanolic, hydro-alcoholic and aqueous extracts had values of 1343.89, 2487.14 and 1154.20 µg $mL^{-1}$, respectively. In the study by Pandaya *et al*. (2013), the *A. indicum* extracts demonstrated a free radical scavenging potential, with IC50 values ranging from 53.08 to 364.94 µg $mL^{-1}$, and the highest free radical scavenging activity was observed in the hexane soluble fraction (IC50= 53.08±0.38 µg $mL^{-1}$), which may be due to its phenolic content, 57.9±0.58 mg of GAE $gm^{-1}$ of extract (Pandaya *et al*., 2013). Although the present findings are similar to previous results, there were differences among the studies in the methods used to extract *A. indicum* and evaluate antioxidant activity as well as the climate, soil, and environmental factors; diseases and pesticide treatments; harvest time; the drying and storage processes; and the plant parts used in the analyses, all of which may have significantly affected the antioxidant activity of the plants (Chakraborthy and Ghorpade, 2010). Several studies have demonstrated the relationship between antioxidant activity and plant phenolic content (Canadanović-Brunet *et al*., 2008), and in the current study, a positive correlation was found between total phenolic content and the antioxidant activity of all the plant extracts.

The main conclusions are as follows. Over the two experimental years of this study, the yield and yield components varied little with higher yields obtained in the first year. Among all of the analyzed extracts (leaf, root and seed), the fertilized plants had higher chemical contents and greater biological activity than the control plants. The CAN fertilizer treatment was particularly effective in terms of improving the yield and yield features, while NPK performed better in terms of the crude oil compositions.

It can be concluded from the present study that root and leaf extracts from *A. indicum* plants exhibit significantly higher phenolic contents and show promising free radical scavenging effects on DPPH in a concentration-dependent manner. In addition, the current findings reconfirmed those of earlier studies that found *A. indicum* to be a reliable natural antioxidant that can safely be used in the pharmaceutical and food industries to prevent the effects of reactive oxygen species and reduce the risks of cardiovascular disease. Thus, the extracts of the roots and leaves of *A. indicum* can play an important role in the prevention of several degenerative diseases, such as hepatic disorders, immune dysfunction, cataracts and macular degeneration, by inhibiting the production of reactive oxygen species, thus reducing the risk of these diseases and promoting proper organ function.

**Resumen**

**G. Yaldiz, A. B.Yildirim, Y. K. Arici y M. Camlica. 2016. Rendimiento, características del rendimiento, composición fitoquímico, actividad antioxidante y antibacteriana del *Abutilon indicum* cultivado con diferentes formas de aplicación de fertilizantes. Cien. Inv. Agr. 43(3):464-475.** El *Abutilon indicum* (Link) Sweet es una importante planta medicinal que se ha empleado durante siglos en la medicina tradicional y una de las novedades en cultivos de la industria farmacéutica. Las prácticas agrarias que producen un alto rendimiento de componentes de plantas secados al aire libre ('drog') y los metabolitos secundarios deseados están muy demandadas por las industrias asociadas. De este modo, el objeto del estudio consistía en determinar el rendimiento de la hoja y la semilla del *Abutilon indicum* Sweet, el rendimiento y composición de su aceite crudo, su contenido fenólico total y su potencial antioxidante y antibacteriano empleando para ello las dos aplicaciones de fertilizante que figuran a continuación: un fertilizante de tres componentes (nitrógeno, fósforo y potasio; NPK, 10-10-40) y nitrato de amonio y calcio (CAN, 26% N). Además, los resultados se han comparado con el grupo de control, sin fertilizante. Los dos experimentos se llevaron a cabo en dos años consecutivos. Los rendimientos más altos tanto de hoja como la semilla se obtuvieron en ambos años con la aplicación del CAN, con unos resultados de 74250.0±5440.0 y 1159.6±62.2 kg $ha^{-1}$, respectivamente. El contenido de aceite crudo de la semilla varió del 13.6 al 14.7%. El ácido linoleico (69.5%) fue el ácido graso predominante en el aceite de la semilla, alcanzando valores mayores con la aplicación del NPK comparado con CAN. Ambos fertilizantes presentaron la mayor actividad antioxidante en las raíces (87.2%). En las hojas, el componente fenólico más alto se observó en la aplicación de control (56.9±0.0 mg GAE $g^{-1}$ extracto seco), y el mayor contenido de flavonoides se detectó en la aplicación del CAN (107.4 mg de pirocatecol $g^{-1}$ extracto seco). En todos los extractos analizados (hoja, raíz y semilla), las plantas fertilizadas presentaron contenidos flavonoides mayores que los obtenidos en la aplicación de control. Además, los extractos de la raíz del *Abutilon indicum* Sweet tuvieron la mejor actividad antibacteriana contra el *Streptococcus pyogenes*.

**Palabras clave:** Aceite crudo, actividad biológica, CAN, fertilizante, NPK, rendimiento.

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