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Influence of Water Hyacinth-Based Organic Manures on Yield and Phytochemical Composition of Cultivated *Cassia angustifolia*

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ABSTRACT

The importance of *Cassia augustifolia* for pharmaceutical industries can not be over-emphasized and ignored. It is imperative to increase yield and bioactivity without the use of harmful chemicals as fertilizers. Thus, this study was undertaken to evaluate the effect of water hyacinth-based organic manure on the growth, yield, and chemical composition of *Cassia augustifolia*. The study employed eight different treatments, each with various water hyacinth and animal waste ratios. Shoot and root length, leaf number, and area were examined to study the morphological growth features. The yield was quantified in terms of the plant's fresh and dry weight recorded at 30, 60, and 90 days after sowing. Standard techniques were used to determine the chemical composition and DPPH radical scavenging activity. The most effective treatment was 25% water hyacinth + 75% chicken droppings, although there was no significant difference in the results compared to 50% water hyacinth + 50% chicken droppings. However, there was no significant difference in using 50% water hyacinth + 50% chicken droppings and 25% water hyacinth + 75% chicken droppings and are the most effective manures. *C. augustifolia* grown on manure with 50% water hyacinth and 50% chicken droppings recorded the highest TPC and TFC (60.82±1.96 mg/g GAE and 135.62±1.99 mg/g QE, respectively). The highest DPPH radical scavenging inhibition (47.83%) was exhibited by C. anugifolia grown on 25% water hyacinth + 75% cow dung manure. Our finding has given scientific insight into the use of water hyacinth-based organic manure to cultivate medicinal plants for optimum yield.

KEY WORDS: CASSIA ANGUSTIFOLIA, DPPH, ORGANIC FARMING, SUSTAINABLE AGRICULTURE, WATER HYACINTH.

INTRODUCTION

The continuous demand and supply has made it necessary to raise the yield of mass produce. Even though conventional farming has led to an increase in the yield of plants but the excessive use of chemical fertilizers has a damaging effect on environment, soil and water (Tsvetkov et al. 2018). Organic manures improve soil fertility, soil structure, water holding capacity, physical and chemical qualities of soil, microbial movement, and supplement accessibility without causing climate change. They likewise upgrade the overall development of the plant. Generally, poultry compost provides the cultivated plants more nitrogen and phosphorus than other natural manures (Garg and Bahl 2008; Vali et al. 2020).

Natural manure upgrades the accessibility of NPK and other fundamental supplements and plays a significant role in the development and advancement of a plant. Natural manure upgrades the vegetative and regenerative development of the plant, for example, Plant tallness, shoots plant, number of leaves, new biomass, and dry biomass (Vidya and Girish 2014; Reddy et al. 2017). Natural compost ensures a significant part in supplement accessibility without having a bothersome impact on Earth. Moreover, organic manures undertake a significant job to upgrade the physical properties of the soil, for example, mass thickness, improve microbial exercises, water retention, and supplement accessibility to the plant (Njoroge et al. 2017; Li et al. 2018; Vali et al. 2020).

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Organically grown medicinal plants are accepted without hesitation and draw a superior value than those grown with conventional farming. The existing data shows organically produced *Cassia angustifolia* leaves are double in price than conventionally grown *Cassia* and pods are as high as forty percent in comparison. Organic manures prepared from water hyacinth have shown to increase soil fertility, yield of the plant and texture of the soil. *Cassia angustifolia* (Senna) belongs to the genus *Cassia* which comprises many other herbs and shrubs. It is a perennial plant with 60 to 80 cm tallness (Jafari 2010; Săvulescu et al. 2018). The plant can grow well in red loamy and alluvial soil with proper drainage of water (Jnanesha et al. 2018; Aishwath and Tarafdar 2020).

It has been used since the early traditional times. Senna leaves are utilized in different conventional frameworks to treat obstruction, loss of craving, hepatomegaly, splenomegaly, acid reflux, and sickliness (Fatima and Girdharilal 2016). Hence, it is necessary to cultivate this plant as an essential medicinal resource which is free from inorganic fertilizers. Inorganic manure stays on the outside of the soil after vast precipitation bringing about draining. It unfavorably influences human health conditions and severely affects individuals' soundness (Sharma and Singhvi 2017). The present study was undertaken to add information to current and limited information on effect of water hyacinth-based organic manures on the yield and chemical composition of cultivated *Cassia angustifolia* as it is meant for direct consumption of humans and also for standard drug preparation.

MATERIAL AND METHODS

For the collection and cultivation of *Cassia augustifolia* seeds, the Zandu Foundation of Health Care in Valsad, Gujarat, provided the *Cassia angustifolia* seeds.

Pot culture experiments were conducted during the 2019 and 2020 monsoon seasons. The experiment was carried out under shade to control excessive exposure to sunlight and rainfall. The soil used in the experiment was a mixture of loamy and sandy soil (3:1 proportion). The experiment was carried out in randomized block design (RBD) with eight treatments and three replicates. The treatments comprised of T1- control (No organic manure); T2- decomposed water hyacinth; T3 (50% Water hyacinth + 50% chicken droppings); T4- (25% Water hyacinth +75% Chicken droppings); T5- (50% Water hyacinth + 50% Goat manure); T6- (25% Water hyacinth + 75% Goat manure); T7- (50% Water hyacinth + 50% Cow dung); T8- (25% Water hyacinth + 75% Cow dung). The organic manures were thoroughly mixed into the pots at a 10t/ha rate and allowed to rest for a few days. *Cassia angustifolia* seeds were planted directly into the pots and allowed to germinate and establish themselves in the soil.

Table 1. Effect of water hyacinth based organic manures on shoot length (cm) of *Cassia angustifolia* during two years

Treatment	Shoot length (cm) Monsoon 2019			Monsoon 2020		
	30 days	60 days	90 days	30 days	60 days	90 days
T1	12.23±0.09^{a}*	15.30±1.65^{a}	28.16±0.60^{a}	9.60±0.70^{a}	12.70±0.94^{a}	26.20±1.54^{a}
T2	12.50±0.81^{a}	15.20±0.43^{a}	32.33±1.59^{ab}	11.43±0.84^{ab}	19.23±1.21^{bc}	30.60±1.46^{b}
T3	12.87±0.65^{ab}	16.86±1.07^{a}	34.67±1.20^{bc}	14.87±1.08^{bc}	23.87±1.05^{cd}	34.27±1.27^{b}
T4	15.10±0.61^{bc}	19.30±1.32^{a}	30.00±1.26^{a}	17.20±0.55^{c}	23.37±1.02^{cd}	30.83±1.07^{b}
T5	15.27±0.12^{bc}	18.00±1.25^{a}	34.67±0.17^{bc}	14.33±0.73^{bc}	24.67±1.02^{d}	33.33±0.44^{b}
T6	15.67±0.88^{c}	19.03±2.51^{a}	37.17±0.44^{c}	15.07±0.52^{c}	17.63±1.91^{b}	32.57±1.49^{b}
T7	14.10±1.16^{abc}	16.43±0.92^{a}	30.17±0.73^{a}	15.10±1.68^{c}	19.90±1.79^{bcd}	33.40±0.93^{b}
T8	13.27±1.12^{abc}	20.33±2.89^{a}	35.50±2.75^{bc}	14.03±1.65^{c}	20.27±0.84^{bcd}	33.53±2.29^{b}

*Values are mean of three replicates ± Standard error of mean
Same letter superscripts down the column denote no significant difference ($p \leq 0.05$)

After emergence, the plant population was reduced to one plant per pot. The growth and yield attributes (root length, shoot length, the number of leaves, and leaf area) and yield attributes (wet weight of the plant and dry weight of the plants) were measured at 30, 60, and 90 days after sowing (DAS). The mature plants from the various treatments were harvested and prepared for further research. For the extraction of Cultivated *Cassia augustifolia* from various treatments, the harvested, washed, air-dried harvested samples were extracted using ethanol via Soxhlelation. The resulting extracts were filtered, dried, and stored for further use. For the phytochemical Composition of *C. augustifolia* from various treatments, the carbohydrate content in *Cassia angustifolia* was estimated using the phenol-sulfuric acid method. The protein rotein content was estimated by the Lowry method (Masuko et al. 2005; Shen 2019). Total phenolic content (TPC) was determined by the Folin-Ciocalteau method, and total flavonoid content (TFC) was estimated by the Aluminum Chloride method (Vijayasekhar et al. 2016; Shen 2019).

Chlorophyll content was estimated using an established protocol. 2, 2-Diphenyl-1-Picryl hydrazyl (DPPH) radical scavenging assay was evaluated using standard procedure (Qaiyum Ansari et al. 2013; Rajalakshmi and Banu 2015; Vijayasekhar et al. 2016). For statistical analysis all the data obtained were subjected to one-way ANOVA, and the means of the treatments were separated and compared using Duncan's Multiple Range Test (DMRT).

Table 2. Effect of Water hyacinth based organic manures on root length (cm) of *Cassia angustifolia* during two years

Treatment	Monsoon 2019			Monsoon 2020		
	30 days	60 days	90 days	30 days	60 days	90 days
T1	4.90±1.35^{a*}	5.46±0.08^{a}	12.90±1.81^{a}	3.00±0.58^{ab}	6.10±0.83^{a}	9.20±0.52^{a}
T2	5.83±0.43^{ab}	7.76±1.64^{ab}	12.07±0.58^{a}	2.40±0.40^{a}	6.03±0.98^{a}	12.23±1.25^{ab}
T3	5.60±0.06^{ab}	9.90±0.30^{acd}	13.13±1.57^{a}	3.76±0.21^{abc}	6.03±1.15^{a}	14.10±0.96^{b}
T4	7.10±0.38^{b}	9.13±0.81^{ac}	15.27±4.11^{a}	5.30±0.53^{bc}	8.53±0.38^{a}	15.67±2.80^{b}
T5	7.50±0.21^{b}	11.17±1.17^{cd}	12.00±1.96^{a}	6.13±0.62^{c}	7.47±0.72^{a}	12.53±1.60^{ab}
T6	7.20±0.46^{b}	11.93±0.52^{cd}	13.03±1.21^{a}	5.40±0.76^{bc}	7.17±0.78^{a}	12.37±0.54^{ab}
T7	5.77±0.24^{ab}	12.03±0.87^{cd}	13.33±0.29^{a}	4.37±0.59^{abc}	6.23±0.58^{a}	12.90±0.49^{ab}
T8	6.03±0.68^{ab}	11.90±0.10^{cd}	12.33±1.13^{a}	4.97±1.57^{bc}	7.33±0.58^{a}	12.93±0.64^{ab}

*Values are mean of three replicates ± Standard error of mean
Same letter superscripts down the column denote no significant difference ($p \leq 0.05$)

Table 3. Effect of Water hyacinth based organic manures on number of leaves of *Cassia angustifolia* during two years

Treatment	Monsoon 2019			Monsoon 2020		
	30 days	60 days	90 days	30 days	60 days	90 days
T1	12.67±1.33^{a*}	36.00±1.00^{a}	72.00±9.23^{a}	6.67±0.67^{a}	17.33±3.17^{a}	43.67±8.09^{a}
T2	14.00±2.31^{ab}	64.67±6.96^{ab}	89.67±9.86^{a}	8.00±1.15^{a}	30.33±2.18^{bc}	62.67±3.76^{a}
T3	15.33±1.76^{ab}	80.33±2.40^{b}	106.33±6.84^{a}	10.67±0.67^{ab}	36.33±0.88^{bc}	97.00±8.33^{b}
T4	13.33±1.33^{ab}	85.33±3.33^{b}	152.67±35.5^{b}	13.67±0.88^{bc}	32.00±4.35^{bc}	157.67±14.17^{c}
T5	19.00±1.00^{b}	71.00±0.00^{ab}	98.33±7.79^{a}	15.00±1.53^{c}	28.33±0.88^{bc}	94.67±6.98^{b}
T6	14.67±2.40^{ab}	75.00±2.00^{ab}	99.67±5.20^{a}	13.67±0.88^{bc}	25.33±3.52^{bc}	101.67±5.55^{b}
T7	16.67±1.76^{ab}	69.67±4.63^{ab}	81.00±5.13^{a}	15.67±1.45^{c}	28.67±2.73^{bc}	91.67±4.48^{b}
T8	13.33±1.76^{ab}	52.00±2.64^{a}	99.00±15.57^{a}	16.00±2.31^{c}	29.67±0.88^{bc}	90.67±4.70^{b}

*Values are mean of three replicates ± Standard error of mean
Same letter superscripts down the column denote no significant difference ($p \leq 0.05$)

RESULTS AND DISCUSSION

The present study results revealed varying morphological characters exhibited by *Cassia augustifolia* under various treatments at 30, 60, and 90 days after sowing (DAS). The shoot length was not much affected by using various water hyacinth-based organic manures (Table 1). However, in the 2019 monsoon season, the highest shoot length was found in treatment T6 (37.17±0.44 cm) 90 DAS. In the 2020 season, T3 (34.27±1.27 cm) had the most extended shoot length, 90 DAS. Conversely, the shortest shoot length for the two monsoon seasons was recorded by T1. Table 2 shows that at the end of 90 days after sowing, treatment T4 (water hyacinth and chicken litter 1:3) shows the highest root length i.e. 15.27 and 15.67 in 2019 and 2020 respectively.

Data in Table 3 shows the effect of organic manures on the number of leaves of *Cassia angustifolia* at 30, 60, and 90 days after sowing. The highest number of leaves were found in treatment T4 (water hyacinth and chicken litter 1:3) during both the harvests i.e. 152.67 leaves/plant and 157.67 leaves/plant in 2019 and 2020 respectively followed by treatment T3 (water hyacinth and chicken litter 1:1) i.e. 106.33 during 2019 and T6 (Water hyacinth and Goat manure 1:3) during 2020. Table 4 shows data of the leaf area of *Cassia angustifolia* for 2019 and 2020 at 30, 60, and 90 days. Data shows that the highest leaf area was observed in treatment T3 (water hyacinth and chicken litter 1:1) during 2019 and T4 (water hyacinth and chicken litter 1:3) during 2020 but the difference between T3 and T4 was not very high in 2019.

Table 4. Effect of Water hyacinth based organic manures on leaf area (cm^2) of *Cassia angustifolia* during two years

Treatment	Leaf Area (cm^2) Monsoon 2019			Monsoon 2020		
	30 days	60 days	90 days	30 days	60 days	90 days
T1	0.90±0.06[a*]	2.42±0.40[a]	4.17±0.80[a]	0.76±0.14[a]	3.02±0.32[a]	3.25±0.37[a]
T2	0.90±0.06[a]	2.57±0.12[a]	4.53±1.59[ab]	2.07±0.68[ab]	3.82±0.34[a]	4.81±0.34[bc]
T3	0.97±0.03[ab]	3.32±0.34[a]	6.45±0.78[b]	4.11±0.34[cd]	4.28±0.52[bc]	4.95±0.21[b]
T4	1.03±0.03[abc]	3.04±0.43[a]	5.75±0.20[ab]	2.75±0.31[d]	4.24±0.13[bc]	6.93±0.06[c]
T5	1.13±0.12[bc]	3.27±0.10[a]	5.94±0.45[ab]	1.99±0.31[abd]	4.03±0.16[ab]	5.76±0.23[b]
T6	1.07±0.07[abc]	2.60±0.08[a]	6.09±0.62[ab]	2.13±0.19[ab]	3.96±0.25[ab]	5.19±0.40[b]
T7	1.20±0.06[c]	2.68±0.41[a]	4.85±0.38[ab]	1.25±0.41[ad]	4.03±0.22[ab]	5.30±0.37[b]
T8	1.07±0.03[abc]	3.08±0.34[a]	4.41±0.50[ab]	1.84±0.25[abd]	3.82±0.41[a]	4.93±0.20[bc]

*Values are mean of three replicates ± Standard error of mean
Same letter superscripts down the column denote no significant difference ($p \leq 0.05$)

Table 5. Effect of Water hyacinth based organic manures on fresh weight of the plant (g) of *Cassia angustifolia* during two years

Treatment	Fresh weight of the plants (g) Monsoon 2019			Monsoon 2020		
	30 days	60 days	90 days	30 days	60 days	90 days
T1	0.86±0.05[a*]	1.83±1.17[a]	4.00±0.58[a]	0.34±0.05[a]	1.70±0.20[a]	2.50±0.29[a]
T2	0.93±0.21[ac]	3.67±0.67[ab]	5.67±0.67[ab]	0.39±0.13[a]	2.34±0.07[ab]	3.50±0.29[ab]
T3	1.13±0.11[abc]	6.00±0.58[cd]	10.00±1.15[c]	2.03±1.68[b]	5.47±0.14[c]	6.00±0.58[cd]
T4	1.44±0.06[bd]	6.33±0.88[d]	13.33±1.85[d]	1.68±0.18[bc]	3.58±0.41[d]	7.00±0.58[d]
T5	1.75±0.13[d]	4.33±0.88[bc]	8.33±0.33[bc]	1.55±0.09[cd]	2.66±0.32[b]	5.67±0.33[c]
T6	1.38±0.03[bcd]	4.67±0.33[bcd]	10.33±0.33[c]	1.32±0.07[cd]	3.11±0.27[b]	6.33±0.33[cd]
T7	1.33±0.20[abcd]	4.17±0.44[bc]	5.67±0.33[ab]	1.49±0.09[cd]	3.65±0.34[d]	4.17±0.17[b]
T8	1.12±0.24[abc]	4.00±0.58[bc]	6.33±1.33[ab]	1.15±0.25[d]	3.76±0.32[d]	3.50±0.29[ab]

*Values are mean of three replicates ± Standard error of mean
Same letter superscripts down the column denote no significant difference ($p \leq 0.05$)

This research shows that treatment T4 (water hyacinth and chicken litter 1:3) showed the highest fresh weight in gram per plant at 90 DAS during both 2019 and 2020, followed by T6. The results of T6 were comparable to that of T3 with proximity. The least effective treatment was T2 which shows that only organic matter supply in the form of water hyacinth was proved to be ineffective when compared with other treatments with a combination of water hyacinth and animal litter in different proportions. Table 6 shows data of the effect of different water hyacinth based manures on the dry weight of *Cassia angustifolia*. Treatment 4(water hyacinth and chicken litter 1:3) shows the best results as far as biomass is concerned, among other treatments. The least dry weight was found in the case of T2 among all other treatments.

The current study was also carried out to determine the quantitative presence of various phytochemicals in *Cassia angustifolia*. The data obtained as mentioned in Figure 1 indicated that carbohydrate and protein content in treatment T7 was the highest among all the treatments (620.52 mg/g DW and 140.78 mg/g DW), while the phenol and flavonoid content (mentioned in Figure 2) was found highest in treatment T3 among others (60.82±1.96 mg/g DW and 135.62±1.99 mg/g DW). Treatment T6 showed the highest chlorophyll a, b, and total chlorophyll content when compared to other treatments as shown in Figure 3 (1.05±0.01 mg/g FW, 0.61±0.06 mg/g FW and 1.67±0.07 mg/g FW respectively). Figure 4 shows that plants treated with treatment T8 showed the best radical scavenging activity (47.83 % at 100 ug/ml concentration of extract).

Table 6. Effect of Water hyacinth based organic manures on dry weight of the plant (g) of *Cassia angustifolia* during two years

Treatment	Dry weight of the plants (g)					
	Monsoon 2019			Monsoon 2020		
	30 days	60 days	90 days	30 days	60 days	90 days
T1	0.26±0.03[a*]	0.43±0.07[a]	1.07±0.23[a]	0.07±0.01[a]	0.32±0.09[a]	0.60±0.06[a]
T2	0.30±0.05[aab]	1.05±0.22[ab]	1.29±0.09[ab]	0.07±0.02[a]	0.79±0.02[b]	1.40±0.21[a]
T3	0.30±0.06[ab]	2.00±0.29[c]	3.00±0.58[c]	0.60±0.01[c]	0.61±0.18[b]	2.83±0.73[bc]
T4	0.42±0.02[abc]	2.08±0.39[c]	3.33±0.33[c]	0.38±0.07[c]	1.07±0.05[bd]	3.00±0.58[bc]
T5	0.57±0.06[c]	1.27±0.37[bc]	2.07±0.07[b]	0.29±0.03[ac]	0.91±0.14[bd]	3.17±0.17[c]
T6	0.46±0.06[bc]	1.33±0.17[bc]	3.30±0.15[c]	0.24±0.11[ac]	1.53±0.20[c]	2.67±0.17[bcd]
T7	0.43±0.06[abc]	1.13±0.18[ab]	1.73±0.09[ab]	0.29±0.03[ac]	1.25±0.03[cd]	1.83±0.12[ad]
T8	0.38±0.09[ab]	1.10±0.21[ab]	1.57±0.33[ab]	0.31±0.26[c]	1.01±0.25[bd]	1.57±0.47[ad]

*Values are mean of three replicates ± Standard error of mean
Same letter superscripts down the column denote no significant difference ($p \leq 0.05$)

Figure 1: Total Carbohydrate (A) and Total Protein contents (B) of *Cassia angustifolia* cultivated with water hyacinth-based manures.

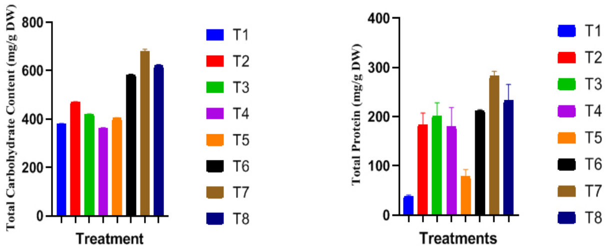
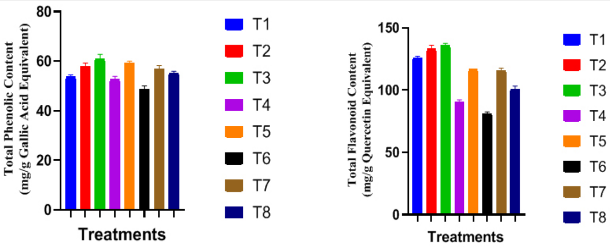
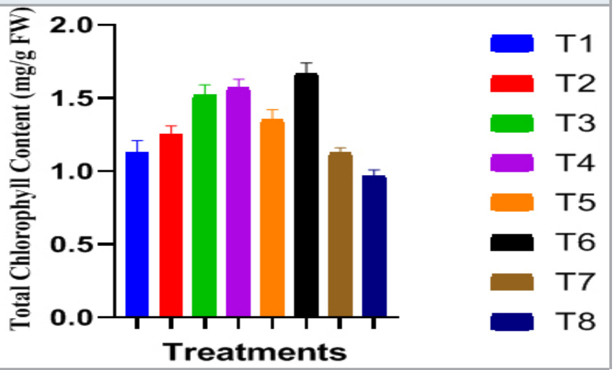


Figure 2: Total Phenolics (A) and Total Flavonoid contents (B) of *Cassia angustifolia* cultivated with water hyacinth-based manures.



Effect of water hyacinth based manures on growth and yield of *Cassia angustifolia*: It was noted that all the treatments produced higher result than control in case of the shoot and root length. Chicken manure and water hyacinth is rich in nitrogen and phosphorus (Amanullah et al. 2007). Hence the combination of the two in equal amounts lead to increased root and shoot length in treatments T3 and T4 as phosphorus is an essential element for root growth and nitrogen is a crucial element for shoot growth as it increases photosynthesis and protein formation which inturn leads to an increased number of leaves as well as leaf area in the plant (Hawkesford et al. 2011; Awad et al. 2012).

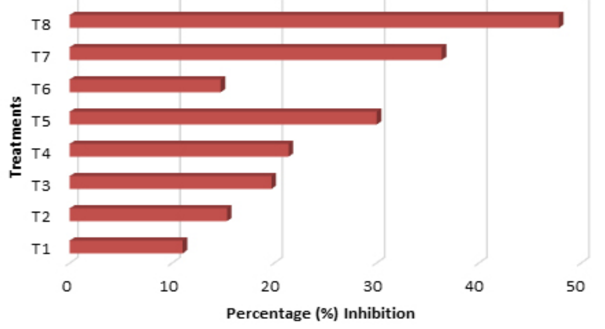
Figure 3: Total Chlorophyll Estimation of *Cassia angustifolia* cultivated with water hyacinth based manures



The increase in these parameters in the case of other treatments was attributed to the factor that the remaining treatments were able to provide nutrients just in a proper amount not exceedingly resulting in no toxicity in any way to the plant. The yield as shown in case of fresh and dry weight of leaves of *Cassia angustifolia* is least in the case of control. Treatment T3 significantly increased the leaf number hence increasing the fresh weight of the plant as well as the dry weight of the plant. Another research study also concluded that the use of water hyacinth-based manures suggestively amplified leaf number. The results of the plant root length in reaction to poultry compost alone or joined with fertilizer is in close concurrence with those revealed by Awad. The primary benefit of using organic manures was to provide plants with nutrients released gradually as the plant grows (Myint et al. 2011; Awad et al. 2012; Moi 2015).

Effect of water hyacinth based manures on phytochemicals of *Cassia angustifolia*: Water hyacinth is utilized as a prospective plant development regulator. These controllers carry out changes in the plant leading to increased production of primary and secondary metabolites. A significant increase in the primary metabolites namely carbohydrate and protein suggest the stable nourishment received by the plants in the form of composts.

Figure 4: DPPH Radical scavenging inhibitory activity of *C. angustifolia* extracts cultivated with water hyacinth-based manures



The secondary metabolites produced by the plants hold a great significance in making medicine from medicinal plants. Higher chlorophyll in treated plants shows balanced nourishment received by the plants. Phenols are commonly found in plants resulting in anti-oxidant properties. The phenolic components of *C. angustifolia* in this study are similar to the reports of Maria (Rani and Usha 2013). Phenols are also considered to have antimicrobial properties. It is evident from the research that *Cassia angustifolia* can be used for its phytochemicals as well as for its potent anti oxidant properties. Flavonoid results agree with the results of past studies (Onofrei et al. 2017). *Cassia angustifolia* has antibacterial, hypo-cholesterolaemic, hepato-protective, anti-diabetic, anti-inflammatory and anti-oxidant actions (Veerabahu and Dec 2010; Silva et al. 2018).

CONCLUSION

The findings of the present study were carried out to explore the potential of different organic manures on the growth and yield parameters of *Cassia angustifolia* M. Vahl. Keeping in view the value of organically grown medicinal plants and due to the increasing use of fertilizers to grow food and medicinal plants, various health issues are reported in humans. Hence, the current study opens up a wide range of possibilities to increase growth, yield and phytochemical components of the plant organically as a part of sustainable agriculture without using chemical fertilizers for the growth of medicinally important plants.

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