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Phytochemical screening and bioremediation using *Ocimum Sanctum* collected from VIT University nursery

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ABSTRACT

The research aimed to find the different phytochemical compounds present in the dry and fresh leaves of Ocimum sanctum and its efficiency in removing chromium. The phytochemical property of Ocimum sanctum was studied using the fresh and dry leaves which was ground using morter and pestle that showed the presence of Carboxylic Acid, phenolic substances, carbohydrates, Reducing sugar, Aldehydes , Ketones ,Esters , Flavonoids and Proteins. After adding of Ocimum sanctum's leaves to the 50 ppm and stems to 25 ppm of chromium solution shows that the Ocimum sanctum has a great adsorbent property as the concentration of chromium decreases with time. Stems are more efficient in removing chromium than leaves. The research can be used for further studies and development of Ocimum sanctum plant for Bioremediation and pharmacological use.

Keywords: Chemical analysis, Bio-remediation

INTRODUCTION

Ocimum sanctum is a short-lived, perennial shrub which is 1m long. It belongs to the family Lamiaceae. It is treated as sacred plant in South Asia as well as medicinal plant in Ayurveda. It is used to treat cold, cough, diarrhea, ulcer, abdominal pain and ulcers. It is also used in anti-cancer, anti-diabetic and anti-fertility drugs. It is mixed with pepper (*Piper nigrium*) and given to pregnant women for treating malaria. It is also used as insect repellant when mixed with grains. It is also used as a flavoring agent in Thai food and is also added to tea. However, Ocimum sanctum can also withstand the large concentration of heavy metals like Chromium and Fluorine in the sample. Chromium exists in the nature in the trivalent and hexavalent form .However, with regards to health hexavalent chromium is more toxic [26,27,28].So, the collected information prompted us to study the phytochemical study and Bioremediation of hexavalent chromium using Ocimum sanctum as the soil in Vellore is Chromium polluted.

MATERIALS AND METHODS

Place of the plant collection:
The Ocimum sanctum leaves are collected at temperature 32^{o}C from VIT University, Vellore Tamilnadu. The pH of the soil was 6.8 .

Fig.1 Ocimum sanctum(Fresh and dried leaves)

Phytochemical Screening:
Ocimum sanctum was ground using mortar and pestle.

Tests for Carboxylic Acids:
Phenolpthalein Test:
To the sample dissolved in 1ml of water one drop of phenolphthalein was added followed by dilute NaOH. Pink colour was produced only after adding number of drops of NaOH indicating the presence of carboxylic acids.[6,8]

Ester formation Test:
Sample was dissolved in 2ml of alcohol and three drops of conc.H_2SO_4 and heated gently for a minute. Then the contents poured into water containing sodium carbonate. Pleasant ester smell was observed confirming that the carboxylic acid was present in the sample.[5,9,12]

Test for phenolic compounds:
Libermann test:
Small quantity of the sample is heated with crystals of sodium nitrite in a dry test tube. Then it was cooled and 2 drops of sulphuric acid was added. The mixture was poured into 100ml of water and added NaOH solution in excess. Solution turned green indicating the presence of phenolic compounds[13,14,15].

Test for carbohydrates:
Molisch's test:
To the sample few drops of alcoholic solution of α-napthol was added.1ml of conc.H_2SO_4 was added along the side of the test tube .Violet ring is formed at the junction which indicate the presence of carbohydrates.[8,9,10].

Benedict's test:
To 5ml of Benedict's solution some amount of sample was added .It was boiled and cooled rapidly. Red precipitate was observed .Thus, showing the presence of reducing sugars.[16,17,18]

Schiff's test:
To the sample few drops of Schiff's base is added. Pink colour developed slowly indicating the presence of aldehydes.[7,19,20,21]

Iodoform test:
To the sample sodium hydroxide solution followed by iodine-potassium iodide solution was added .The solution is warmed on a water bath at 60^oC for 20 mins. Pale yellow precipitate was observed which indicate the presence of ketones in the sample.[22,23,24]

Test for esters:

Hydroxamic Test:

To the sample few drops of hydroxylamine hydrochloride and about 10% sodium hydroxide solution was added and the mixture was gently boiled for 2mins.It was cooled and acidified with dil. HCl and then few drops of $FeCl_3$ was added .Deep brown colour developed indicating the presence of ester.[1,2,3,4,25,26]

Tests for flavonoids:

Test for alkaline reagent:

To the sample few drops of NaOH was added, the colour developed turns to be colorless after the addition of dil. Acetic acid indicating the presence of flavonoids in the sample [1,2,3,4].

Ferric chloride test:

Few drops of ferric chloride was added to the sample. Green colour was formed indicating the presence of flavonoids.[11]

Bioremediation of Chromium using Ocimum sanctum:

25 ppm and 50 ppm concentration of chromium was made using K2Cr2O7. 5gm of Ocimum sanctum's leaves and stems were added to the 25 ppm and 50 ppm chromium solution in different conical flasks. Sample were collected after 15mins, 30mins, 75mins and 120mins.The samples were analysed using Atomic Absorption Spectroscopy. The efficiency of sample in removing chromium was found out using the formulae:

% removal=concentration at time(t=0)-concentration at time (t=t)/concentration at time(t=0)*100

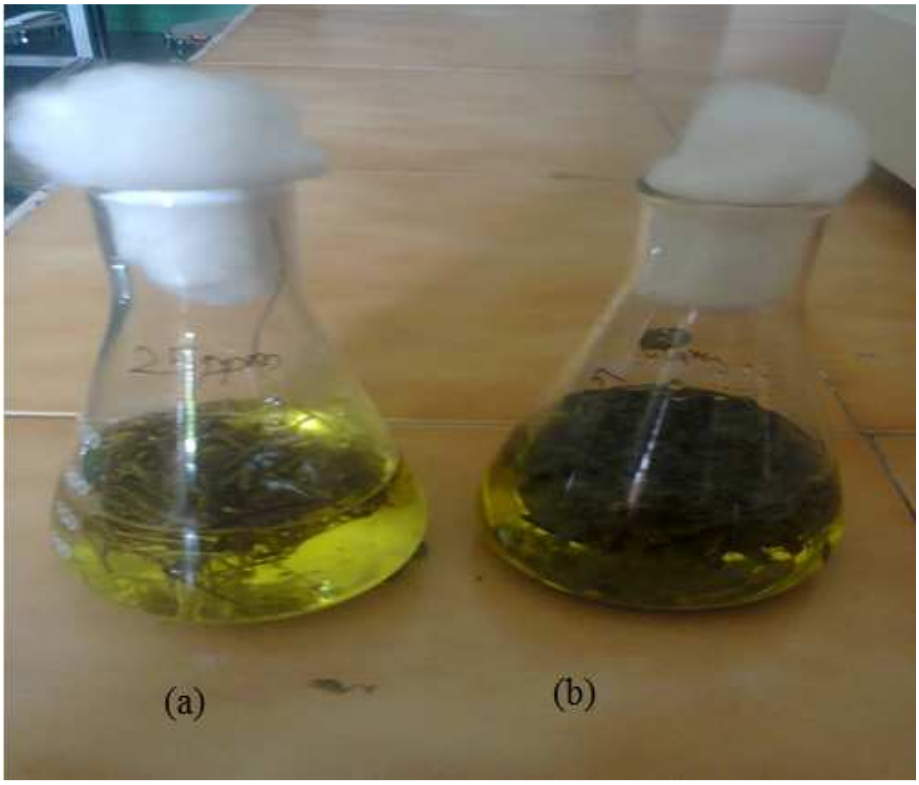

Fig2. Initial concentration of Chromium :(a)leaves (b)stems

RESULTS AND DISCUSSION

Fig.3: Samples collected after intervals of time: (a)leaves (b)stems

Table 1: Concentration of chromium in collected sample after intervals of time

Time after adding Sample	Leaves(initial conc.of chromium 50ppm)		Stems(initial conc.of chromium 25ppm)	
	Conc.mg/l	Absorbance	Conc.mg/l	Absorbance
15mins	42.607	0.0088	19.763	0.0063
30 mins	39.988	0.0082	17.217	0.0055
24 hours	24.952	0.0046	9.611	0.0031
48 hours	21.713	0.0041	7.642	0.0024

Table 2: Percentage of removal of chromium with time

Time	Dry leaves (%)	Stems(%)
15mins	14.78	20.094
30mins	20.02	31.132
24 hours	50.176	61.556
48 hours	56.676	69.432

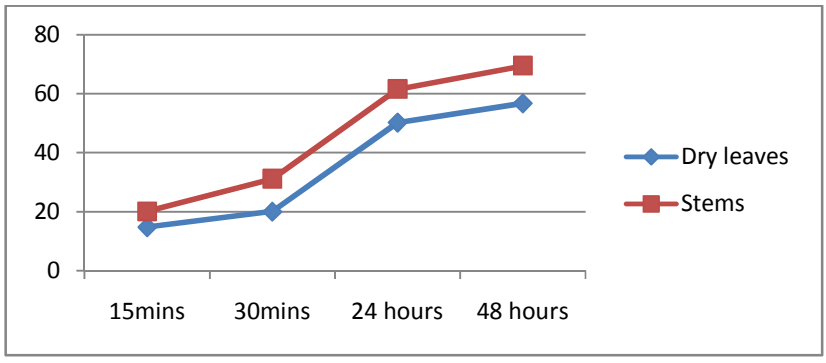

Fig.4: Efficiency of dry leaves and stems in removing chromium

The results shows that the Ocimum sanctum has a great adsorbent property as concentration of chromium decreases with time and Stems are more efficient in removing Chromium than the Dry leaves. Therefore, Ocimum sanctum poses stimulant to enhance bioremediation of chromium polluted water.

Table 3: Phytochemical Screening

S.No	Name of Test	Procedure	Observation	Inference	Sample	
					Dry leaves	Fresh Leaves
1.	**Test For Carboxylic Acid:**					
	Phenolpthalein test	Sample+1 drop of phenolphthalein+dil.NaOH	Pink colour	Presence of Carboxylic Acid	+ve	+ve
	Ester Formation test	Sample+2ml of alcohol+3 drops of conc.H_2SO_4 +heat for 1 min+contents poured into water containing Na_2CO_3	Pleasant ester smell		+ve	+ve
2.	**Test for Phenols**: Libermann test	Sample+sodium nitrite+2 drops of H_2SO_4 +100ml of water with excess NaOH	Solution turning green	Presence of Phenols	+ve	+ve
3.	**Test for Carbohydrates:**					
	Molisch's test	Sample+alcoholic solution of α-napthol+1ml of conc.H_2SO_4 along the side of the test tube	Violet ring is formed at the junction	Presence of Carbohydrates	+ve	+ve
	Benedict's test	5ml of Benedict's soln.+sample boiled and cooled rapidly	Red precipitate	Presence of Reducing Sugar	+ve	+ve
	Schiff's test	Sample+ few drops of Schiff"s base	Pink colour developed slowly	Presence of aldehydes	+ve	+ve
	Iodoform test	Sample+NaOH+iodine-pottasium iodide+heat in water bath for 20 mins	Pale yellow precipitate	Presence of ketones	+ve	+ve
4.	**Test for Esters:** Hydroxamic test	Sample+hydroxylamine hydrochloride+ 5ml of 10% NaOH .Heat it gently for 2 mins .Cool it and acidify with dil.HCl+ few drops of $FeCl_3$	Deep red brown colour	Presence of Esters	+ve	+ve
5.	**Test for Flavonoids:**					
	Alkaline reagent test	Sample+Few drops of NaOH soln.	Colorless	Presence of Flavinoids	+ve	+ve
	$FeCl_3$ test	Sample+ few drops of $FeCl_3$ soln.	Green colour		+ve	+ve

The chemical analysis of the sample shows that Ocimum sanctum's dry leaves and fresh leaves both contains Carboxylic Acids ,Phenolic substances, Carbohydrates, Aldehydes, Ketones, Esters, Flavonoids and Proteins. This research can be useful for further studies and development of Ocimum Sanctum for pharmacological and Bioremediation use.

Fresh Leaves:

Fig.4: Chemical analysis of fresh leaves

Dry Leaves:

Fig.5: Chemical analysis of dry leaves

CONCLUSION

From the above study it is concluded that Ocimum Sanctum can be used for bioremediation of hexavalent chromium as it has great adsorbent property. However, stem was found to be more efficient in removing Chromium than leaves. Stems removed 69.432% and leaves removed 56.676 % of chromium in two days. It also contains phytochemical compounds like Carboxylic Acid, Phenols, Carbohydrates, Aldehydes, Ketones , Esters and Flavonoids. Thus, it can be used for pharmacological use.

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REFERENCES

[1] Suneetha V., Bishwambhar Mishra, Parul Kamat, Gopi chand,T., Saranya C., Rani Anupama, Alok Prakash, *Applied Mathematical Sciences*. **2013**, 7, 1563 – 1567.
[2] Suneetha Vuppu and Bishwambhar Mishra, *Der Pharmacia Lettre*, **2013**, 5, 100-106.
[3] Jai Prakash Singh, Satish k. Singh, Ruchika Chandel, Bishwambhar Mishra, Suneetha V . *Int. J. Pharm. Sci. Rev. Res.*,14, 72-76.

[4] Alok Prakash, Kanupriya Mathur, Ankita Vishwakarma, Suneetha Vuppu, Bishwambhar Mishra , *Int. J. Pharm. Sci. Rev. Res*., 19, 131-135.
[5] Sanjeeb Kumar Mandal And Suneetha Vuppu, *Int J Pharm Bio Sci*. **2013**, 4,193 – 200.
[6] Saranya Chitturi, Venkata Gopichand. Talatam and Suneetha Vuppu, Der Pharmacia Lettre,**2012**,5, 13-23.
[7] Sanjeeb Kumar Mandal, M. Vignesh Kumar, Suneetha V , *Int. J. Pharm. Sci. Rev. Res.*, 19, 114-118.
[8] Saranya Chitturi, Venkata Gopichand Talatam and Suneetha V , *Int. J.Drug Dev. & Res*.**2013**, 5,131-137.
[9] Siddharth Sharan, Naina Thangaraj, Bishwambhar Mishra, Suneetha V . *Int. J. Drug Dev. & Res*.,**2013**, 5, 138-144.
[10] Sonika Jha , Vineet Vaibhav and Suneetha V. *Int. J. Drug Dev. & Res*.,**2013**, 5, 362-367.
[11] Vaishnavi R and Suneetha V. *Der Pharmacia Lettre*,**2013**, 5, 71-75.
[12] K. Lohitesh, Alok Kumar Behera, Allen Abey Alexander and Suneetha V, *Der Pharmacia Lettre*, **2013**, 5, 163-169.
[13] Amit Kumar Srivastav, Padmakshi Agarwal, Kaushik Jyoti Talukdar and Suneetha Vuppu, Der Pharmacia Lettre,**2013**, 5, 481-491.
[14] Suneetha V, Bishwambhar M, Gopinath R.,Shrestha S R, Kartik G K.B., Pravesh C, Apoorvi C, Kalyani R, *Asian Journal of Microbiology Biotechnology and Environmental Sciences*,**2013**,14,405-412.
[15] Suneetha Vuppu, Bishwambhar Mishra and Ching Lee, *International Journal of Pharmaceutical Sciences and Research*,**2013**,3,4242-4246.
[16] Suneetha V and Raj V .*International Journal of Drug Development & Research*,**2012**, 4, 1-6.
[17] Bishwambhar Mishra and Suneetha Vuppu, *Asian Journal Of Microbiology Biotechnology & Environmental Sciences*,**2012** ,14,369-374.
[18] Bishwambhar Mishra and Suneetha V, *International Journal of Drug Development & Research*.**2012**, 4, 259-262. .
[19] Singh Sanjay, Amod Kumar, V Suneetha, Bishwambhar Mishra, Gopinath R, Sharad Yadav and Bhaskar Mitra, *International Journal of Drug Development & Research*, **2012**, 4, 23-30.
[20] Ritika S, Abhishek G, Rahul G, Suneetha V. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*,**2012**, 3, 40-48.
[21] Gopinath R. and Suneetha V. , *Research Journal of Pharmaceutical, Biological and Chemical Sciences* ,**2012**,3, 484-488.
[22] Rai S, Mehrotra S, Dhingra D, Prasad M,. Suneetha V. *International Journal of Pharmaceutical Sciences Review and Research* ,**2012**,17, 40-45.
[23] Bera, A., Verma, S., Suneetha, V *Der Pharmacia Lettre*,**2013** 5 , 58-64.
[24] Lohitesh, K., Behera, A.K., Alexander, A.A., Suneetha, V. *Der Pharmacia Lettre*,**2013**, 5, 163-169
[25] Vaishnavi, R., Suneetha, V. *Der Pharmacia Lettre* , **2013**, 5, 71-75.
[26] Srivastav, A.K., Agarwal, P., Talukdar, K.J., Vuppu, S. *Der Pharmacia Lettre*,**2013**, 5 , 481-491.
[27] Revathi, K., Singh, S., Azeem Khan, M.,Suneetha, V. ,*International Journal of Pharmaceutical Sciences Review and Research* , **2013**, 20, 89-92.
[28] Sai Shiva Shankar, V., Suneetha V. **2013** *International Journal of Pharma and Bio Sciences*,2013, 4 , 795-802