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Combining Studies on Preparation of Metal Nanoparticles from Plant Extracts as Nano-Antibiotic

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Abstract: ***Nano antibiotics are an arising antibiotics particularly arranged for the multidrug resistance, the infection basically occurred by the medically valuable strains that have genetically altered and become unaffected to the antibiotics. The application of ions of metals and salts being unchanged but precisely a affect the bacterial resistance mechanisms. The plants contain antimicrobial activity can be employed for the complementary therapies as stuff for the nanoparticle creation. The naoparticles have powerfuldestination action on the pathogen as its working efficiency can be altered as per requirement. The naoparticles can be used as tablets and also in suspensions. The multi drug resistantpathogens would be hindered and its strain could be altered for our aim. The employment of alteredspecimen with the salts can be grouped as per the wanted dosage for the bacterial pathogens.***

Keywords: Nanoparticles, Nanoantibiotics, Phytocompounds, Phytochemicals, Plantshifts

1. Introduction

Plants are the major source of the phytocompounds which shows many medicinal properties. These compounds help to hinder challenger, predators or pathogens. The name originates from Greek, where phyto means plant. Some of the phytochemicals employed as toxins and others as common drugs. Phytochemicals is broadly employed to explain shifts of plants that are under analysis with well-established results on health and are not scientifically explained as essential nutrients. Phytochemicals under analysis can be classified into major divisions, such as carotenoids and polyphenols, which carry phenolic acids, flavonoids, and stibenes/lignans. Flavonoids can be grouped as epicatechins, catechins, and proanthocyanidins. Phytochemicals acquired from fruits ,vegetables, spices, herbs and medicinal plants, such as, terpenoid, alkaloids and other phenolic stuffs, have been accessed to hinder developmental tumor genesis in different organs in pre-clinical models. Most of oxidation inhibition activity is ascribed to the flavones, isoflavones, flvonoids, anthocyanin, coumarin, catechin etc. oxidation inhibitory based drug formulations are serviced for the avoidance and treatment of complex diseases like stroke, diabetes,Alzheimer's disease and cancer. The biological molecules those are present in the plants shifts are serviced to minimize metal ions to nanoparticles in a single-step green synthesis process. This biogenicdevaluation of metal ion to base metal is absolutely accelerated, immediately managed at room temperature and pressure, and comfortably scaled up. Synthesis conciliated by plantshifts is environmentally benign. The reducing agents involved the various solvent or water soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Extracts of a diverse range of plant species have been successfully used in making nanoparticles.

2. Material and Methods

2.1 Sample collection

The plant samples were collected and then separated into different parts of the plants

Table 1: Three different samples plant samples were collected

S no.	Scientific name	Common name	Location
1.	*Calendula officinalis*	Marigold orange	Gomti Nagar, Lucknow (UP)
2.	*Calendula officinalis*	Marigold yellow	Gomti Nagar, Lucknow (UP)
3.	*Matricariachamomilla*	Chamomile	Gomti Nagar, Lucknow (UP)

2.2 Microbial strain and culture preparation

Salmonella typhi(*St*), *Pseudomonas aeruginosa* (*Pa*) and *Bacillus subtilis (Bs)*are some bacterial pathogens which are available at the MRD LifeSciences(P) Ltd. Lucknow. The pathogens were subcultures and then broth was prepared for further testing's.

2.3 Extract preparation

The samples were separated in different parts of plants, and then properly washed with double distilled water then air dried. For organic extract preparation the samples were dipped in polar and nonpolar solvents in the ratio of 1:10, and incubated at room temperature for 48-96 hours. For aqueous extract preparation the sample were boiled in same ratio at 75℃-100℃ for 90 minutes. Then the extracts were filtered and collected for nanoparticles preparation.

2.4 Nanoparticle preparation

The nanoparticles preparation was based on bottom up synthesis approach, where the plant extracts and the metals in 100mM concentration are taken in three different ratios, such as 1:1, 1:4 and 4:1 at 80°C in hot plate magnetic stirrer. The confirmation is done by the color change of the sample extract. The nanoparticles were incubated for drying and then collected for further use.

2.5. Antibiotic Sensitivity Tests:

The test is based on agar well diffusion method, where the pathogens were firstly spread over the sterilized nutrient agar media and the samples were loaded to the well. Incubated at 37°C for 24 hours and then zone of inhibition was calculated.

2.6. Minimum Inhibitory Concentration test:

The prepared nanoparticles were serially diluted in sterilized nutrient broth and then the bacterial pathogens were inoculated to the respective test tubes. Incubated at 37°C for 24 hours at 121 rpm and then OD was taken at 620 nm.

2.7 Phytochemical Analysis:

a) Flavanoids
- 10% lead acetate/ lead nitrate was prepared.
- 1 ml of extract was added with 1 ml of lead nitrate.
- A yellow precipitate was observed that determined positive result for flavonoids.

b) Saponin
- 1ml of extract with 3 ml of distilled water was taken, mixed.
- Froth denotes positive result for saponin

c) Tannin
- Few drops of lead nitrate was added in 1 ml of extract.
- Precipitate was observed for positive result.

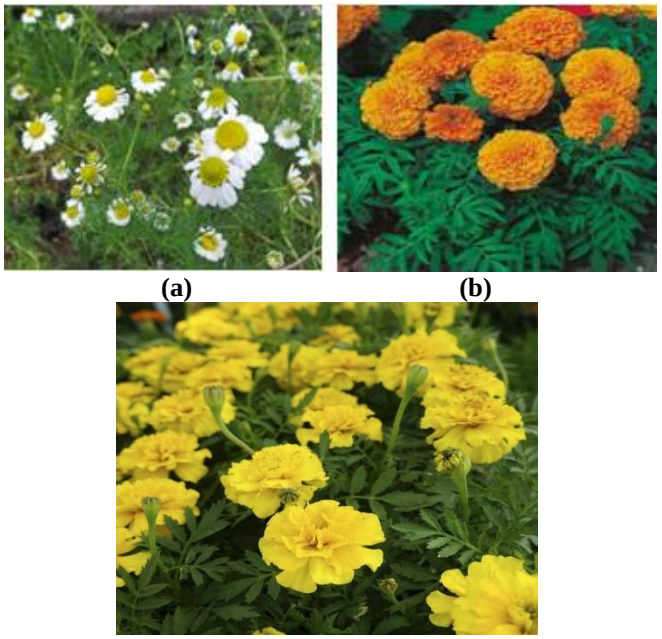
d) Steroids
- 1 ml of extract was added with 2 ml of Chloroform and 2 ml of H2SO4.
- Reddish brown interface shows the positive result.

e) Terpenoids
- 0.1ml of chloroform was added with 0.1 ml of extract.
- 0.1ml of H2SO4.
- Few drops of acetate shows Red color indicating positive result.

f) Carbohydrates
- 0.1ml of extract was added with 0.1 ml of Fehling A.
- 0.1 ml of Fehling B was added in the solution.
- A red precipitate indicates positive result.

3. Results

3.1 Sample Collection



(a) **(b)** **(c)**

Figure 1: (a)*Matricaria chamomilla* **(b)***Calendula officinalis (C)Calendula officinalis*

3.2 Antimicrobial sensitivity test of nanoparticles of aqueous extracts of samples

Table 2: Antimicrobial test of the nanoparticles against the P. aeruginosa, S typhi andB. subtilis

S no.	Nanoparticles	Zone of Inhibition (mm)		
		P. aeruginosa	*S typhi*	*B. subtilis*
1	***Calendula officinalis* (Yellow Flowers)- Flowers**			
a	$ZnSO_4$ (1:1)	30	32	29
b	$FeSO_4$(1:1)	22.5	22.5	26.5
c	$CuSO_4$(1:1)	30	24	24.5
2	***Calendula officinalis* (Yellow Flowers) - Roots**			
a	$ZnSO_4$ (1:1)	29	30	34
b	$FeSO_4$ (1:1)	26.5	27	25.5
c	$CuSO_4$ (1:1)	13	15	12.3
3	***Calendula officinalis* (Yellow Flowers)- Stems**			
a	$ZnSO_4$ (1:1)	23	21.2	14.2
b	$FeSO_4$ (1:1)	18	17.4	14.9
c	$CuSO_4$ (1:1)	20.2	29	30
4	***Calendula officinalis* (Orange Flowers)- Flowers**			
a	$ZnSO_4$ (1:1)	19.7	20.6	21.1
b	$FeSO_4$ (1:1)	16.9	13	14
c	$CuSO_4$ (1:1)	24.5	19.8	20.5
5	***Calendula officinalis* (Orange Flowers)- Roots**			
a	$ZnSO_4$ (1:1)	12.3	21	13.9
b	$FeSO_4$ (1:1)	22	19	14
c	$CuSO_4$ (1:1)	31	12	23
6	***Calendula officinalis* (Orange Flowers)-Stems**			
a	$ZnSO_4$ (1:1)	14.5	13.1	20.1
b	$FeSO_4$ (1:1)	18.6	19	13
c	$CuSO_4$ (1:1)	20	21	19
7	***Matricaria chamomilla* Flowers**			
a	$ZnSO_4$ (1:1)	20	23	21
b	$FeSO_4$ (1:1)	23	29.1	28
c	$CuSO_4$ (1:1)	28.1	26	21
8	***Matricaria chamomilla*Stems**			

a	$ZnSO_4$ (1:1)	13.2	17.5	18
b	$FeSO_4$ (1:1)	21	20	22
c	$CuSO_4$ (1:1)	23	24.2	22
7	***Matricaria chamomilla* Roots**			
a	$ZnSO_4$ (1:1)	15	13.4	19
b	$FeSO_4$ (1:1)	19	18	22
c	$CuSO_4$ (1:1)	23.4	25.6	22.5
8	**Controls**			
a	Norflox	13	11	10.9
b	$ZnSO_4$	11	12.4	13.5
c	$FeSO_4$	10.9	14.3	13.1
d	$CuSO_4$	15	11	10.8

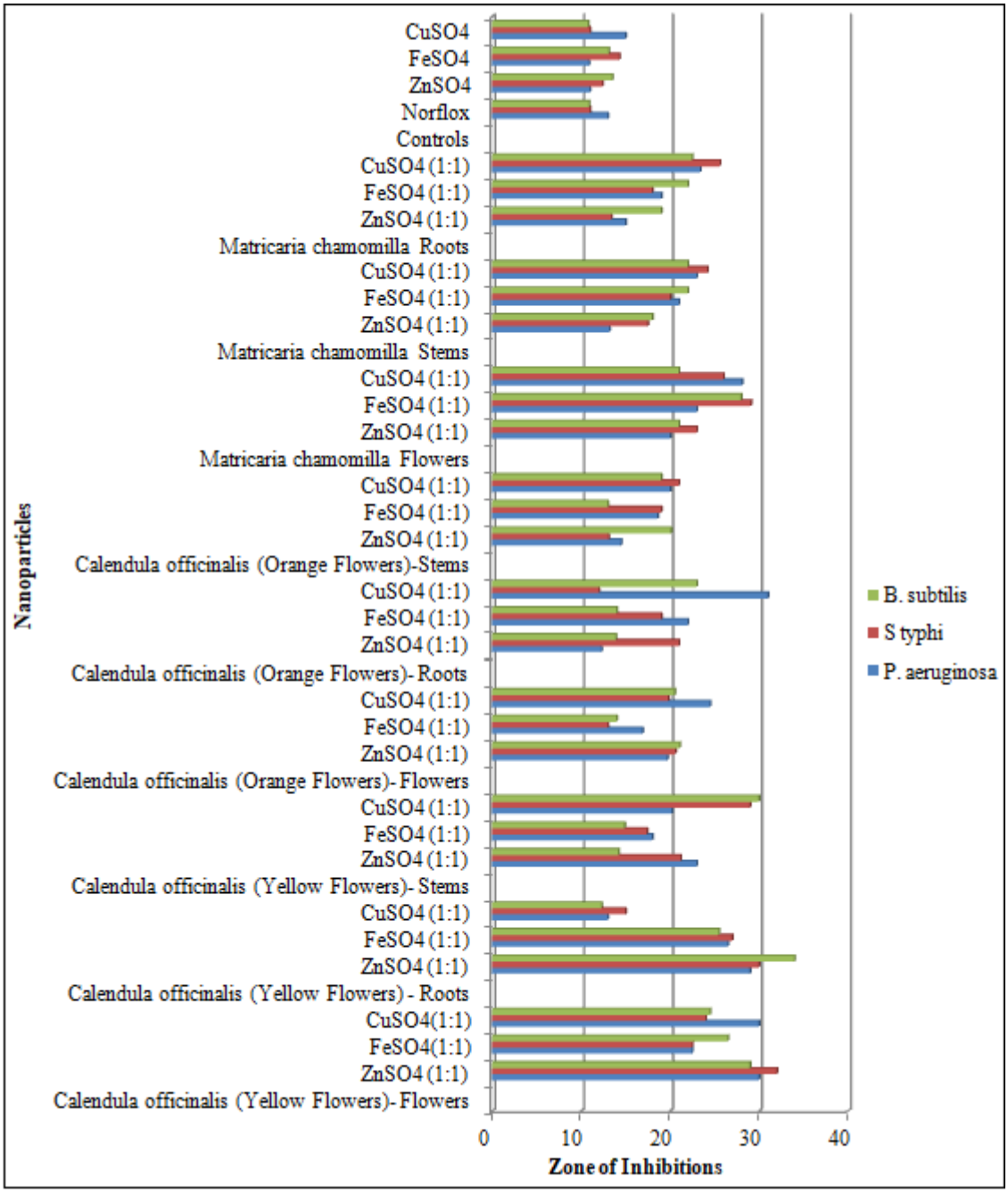


Figure 2: Graphical representation of the antimicrobial test for nanoparticles against bacterial pathogens

3.3 Minimum Inhibitory Concentration Test

Table 3: MIC value calculated for nanoparticle of calendula officinalis (yellow flower), of calendula officinalis(orange flower), Matricaria chamomilla flower against B subtilis

S no.	Nanoparticles	MIC (mg/ml)
1	***Calendula officinalis* (Yellow Flowers)- Flowers**	
A	$ZnSO_4$ (1:1)	14
B	$FeSO_4$ (1:1)	10
C	$CuSO_4$ (1:1)	15
2	***Calendula officinalis* (Yellow Flowers) - Roots**	
A	$ZnSO_4$ (1:1)	21
B	$FeSO_4$ (1:1)	24
C	$CuSO_4$ (1:1)	12
3	***Calendula officinalis* (Yellow Flowers)- Stems**	
A	$ZnSO_4$ (1:1)	13
B	$FeSO_4$ (1:1)	21
C	$CuSO_4$ (1:1)	30.1
4	***Calendula officinalis* (Orange Flowers)- Flowers**	
A	$ZnSO_4$ (1:1)	14.2
B	$FeSO_4$ (1:1)	19.1
C	$CuSO_4$ (1:1)	15.9
5	***Calendula officinalis* (Orange Flowers)- Roots**	
A	$ZnSO_4$ (1:1)	23
B	$FeSO_4$ (1:1)	21
C	$CuSO_4$ (1:1)	24.1
6	***Calendula officinalis* (Orange Flowers)-stems**	
A	$ZnSO_4$ (1:1)	32.1
B	$FeSO_4$ (1:1)	22
C	$CuSO_4$ (1:1)	18
7	***Matricaria chamomilla* Flowers**	
A	$ZnSO_4$ (1:1)	10.7
B	$FeSO_4$ (1:1)	13
C	$CuSO_4$ (1:1)	15.4
8	***Matricaria* chamomilla Stems**	
A	$ZnSO_4$ (1:1)	21.1
B	$FeSO_4$ (1:1)	28.9
C	$CuSO_4$ (1:1)	31.9
9	***Matricaria chamomilla* Roots**	
A	$ZnSO_4$ (1:1)	12.2
B	$FeSO_4$ (1:1)	15.8
C	$CuSO_4$ (1:1)	28.3

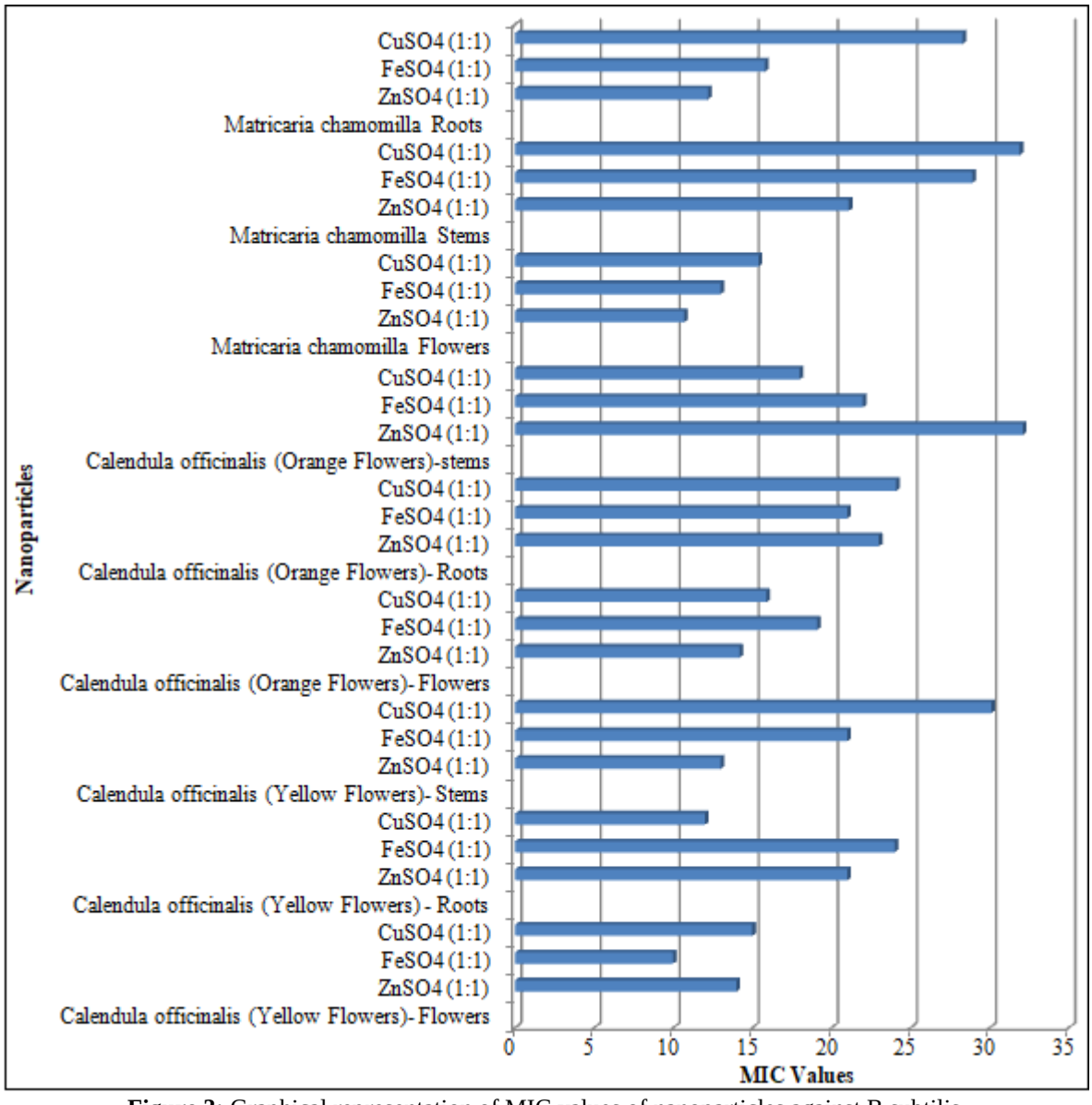


Figure 3: Graphical representation of MIC values of nanoparticles against B subtilis

3.4 Phytochemical Analysis

Table 4: Test for different phytochemical analysis was done and results were observed.

S no.	Samples	Steroids	Flavonoids	Tannin	Terpenoids	Carbohydrate	Saponin
1.	*Calendula officinalis* (Yellow Flowers)- Flowers	+	+	+	+	+	+
2.	Calendula officinalis (Yellow Flowers) - Roots	-	-	-	+	+	+
3.	Calendula officinalis (Yellow Flowers)- Stems	+	+	-	-	+	-
4.	Calendula officinalis (Orange Flowers)- Flowers	-	-	-	-	+	-
5.	Calendula officinalis (Orange Flowers)- Roots	-	-	-	+	+	+
6.	Calendula officinalis (Orange Flowers)- Stems	-	-	+	+	+	+
7.	Matricaria chamomilla Flowers	+	+	+	+	+	+
8.	Matricaria chamomilla Roots	-	-	-	-	+	-
9.	Matricaria chamomilla Stems	+	-	+	-	+	-

4. Discussion

Nanotechnology can be explained as technology that can overwhelm the simple mechanisms of drug release and modified antibiotic work action that would aid to kill the mutated bacteria. The sample used Marigold orange, Marigold yellow, and Chamomile have all important photochemical that hinder the growth of pathogens.Metal Nanoparticles prepared by $ZnSO_4$ and flower of Marigold yellow against gave best results*S.typhi* .The nano- drug creation was done with preparing various concentrations of the salts of metals combined with various concentrations with the desired extract and hold for incubation of 24hrs . the shifts and the metal ions after reaction were then admitted for magnetic stirrer methods and centrifuge method. The magnetic stirrer method was fruitful as it employed rotations for time period also the specimen was heated, the color alteration before and after the use of magnetic stirrer expressed the increased kinetic energy and so the color alterationdepicts the precipitation of the nano particles in the extract. The discriminative studies of the best concentrations of our antibiotic were examined with the marketed drugs. One of which overcome the market drug.

5. Conclusion

The multi drug resistance are a new complication basically in the medical sectors, the probability of the infections are huge there. The newly altered and developed pathogens have the tendency to unaffected fromvarious antibiotics. The entry of the antibiotics is made easy with the tiny sized particles. The employment of compound that has antimicrobial activity can be employed also its working efficiency can be intensified. The nano particles were constructed through magnetic stirrer methods. The application of these methods was to easy to examine the preparation of nanoparticles. The assessment was analyzed by using the antimicrobial susceptibility test of the prepared nanoparticles. The best concentrations were analyzed against with the marketed drugs. Prepared nanoparticle drug defeated norfloxcin and gave best ZOI against the pathogen .The MIC also depicted the better concentrations against pathogens. The medically essential strains are susceptible to the phytochemicals that's why sample were analysed for the phytochemical tests.

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References

[1] G.Sethilmurugan, Vasanthe and Suresh, K.2013. Screening and antibacterial activity analysis of some important medicinal plants.Int.J. Innovation and pharmaceutical research & applied studies. Vol.2. 2feb pp.146-152

[2] Dash, B. K. Faruquee, H M, Biswas S K, Alam, M K Sisir, S.M andProdhan U K 2011. Antibacterial and antifungal activities of several extracts of Centrellasistica L. against some pathogenic microbes. Life sciences and medicine research.

[3] Gao B, Chen Y, Zhang M U and Pan S.2011. Chemical composition,antioxidant and Antimicrobial Activity of PericarpiumCitriReticulatae Essential Oil Molecules.16: 4082-4096.

[4] Hulya H, Demirci A S,Gumum T.2011. Antibacterial and antifungale Activity of Pomerranate (Punicagrantum L.CV.) peel.ElectronicJ.environmental,agri. Food chem. 10(3);1958 -1969.

[5] Makhija I K, DevangKhamar, 2010. Phytochemical and Pharmacological properties of Ficusreligiosa: an over view. Scholars research library. 1(4): 171-180.

[6] Kumar V R, ShashidharaN S and Anita S.2010.Anti typhoid activity of aqueus extract of fruit peel Citrus sinesis(L).Int,J.PharmaReseach Development 2(9);217-221

[7] Mohamed Z. M. Salem, 2013. Antimicrobial activities and phytochemical composition of extract of Ficus species. An over view. African Journal of Microbiology Research. Vol. 7(33), pp, 4207-4219.

[8] Momo I. T, Kuete V, Dufat H, 2011. Antimicrobial activity of the methanolic extract and compounds from the stem brak of Garcinia Lucida Vesque (Clusiaceae). Int J Pharm Sci, 3(3); 215-217.

[9] Kumar M, Nehra K, 2012. Phytochemical analysis and antimicrobial efficacy of leaf extracts of PithecllobiumDulce.Asian journal of pharmaceutical and clinical research. Vol 6. ISSN- 0974-2441.
[10] Kumar M, Nehra K, 2014. Antimicrobial activity of crude extracts of pithecellobiumDulce bark against various human pathogenic microbes. World journal of pharmacy and pharmaceutical sciences. Voi. 3, issue 5, 1244-1260.
[11] Ratherb R A,Swethia C, 2010. Screening of peel extract as antioxidants, anticancer agents and antimicrobials. Advances in bioresearch 1 (1); 29-33.
[12] Sharma M C and Sharma S. 2010. Phytochemical screening and in vitro Antimicrobial activity of combined Citrus paradsi and Ficuscaricalinn aqueous extract int. J. microbiological research 1 (3): 162-165.
[13] Swaati S, Nitika V, 2014. Screening of phytochemical constituents of hydro-ethanolic extracts of aerial parts of Pithecellobiumdulce and Ricinuscommunis. Research journal of chemical sciences. Vol. 4(8), 54-57.
[14] Shihabudeen H M S, 2010. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. Int. J. Pharma Sci. and Research 1(10): 430-434.
[15] Singh S. 2010. In vitro antifungal activity of some essential oils against food spoilage fungi. J. Herbal medicine and Toxicology 4(2): 107-111.
[16] Uchechi N. 2010. The antibacterial activity of crude leaf extract of Citrus sinensis. J. Pharma and Bio sci. 1 issue-4, 742-750.
[17] Nitika V, Swaatim S, 2015. Evalution of RicinusCommuns(L) and PithecellobiumDulce activities against some common human pathogenic bacterial and fungal microbes. Universal journal of Pharmacy.ISSN 2320-303X.
[18] Zu Y, Yu H, Liang L, Efferth T. 2010.Activityes of Ten Essential Oills toward Propionibacterium acnes and PC-3, A-549 and MCF-7 Cancer Cells. 15:3200