International Journal of Advance Research in Science and Engineering
Vol. No.5, Issue No. 03, March 2016
www.ijarse.com
IJARSE
ISSN 2319 - 8354

ANTIMICROBIAL AND ANTIHELMINTHIC ACTIVITY OF APRICOT & ORANGE PEEL BY UNKNOWN BACTERIA

Deepesh Kumar[1], Vivek Mishra[2], V.K.Srivastava[3], Shaurabh Singh[4], Shivani Shukla[5], Anshu Raj Verma[6]

[1,2,3,6] Post Graduate, Department of Biotechnology, [4]Post Graduate Department of Food Technology, RBS Engineering Technical Campus, Bichpuri, Agra, AKTU Lucknow, (India)

[5]Department of Bioengineering, Integral University, Lucknow, (India)

ABSTRACT

Apricot belongs to the family of citrus. Apricot is rich source of vitamin C (Ascorbic acid) and Orange is also possesses maximum quantity of vitamin C (Ascorbic acid). In the present study the antimicrobial activity aqueous, ethanolic and methanolic extract which indicate the maximum antimicrobial activity shows in Orange ethanolic extract in Staphylococcus, apricot methanolic extract shows maximum antimicrobial activity in 10% concentration in the presence of Staphylococcus, in orange methanolic extract 30% shows maximum antimicrobial activity in the presence of Enterococcus bacteria and in apricot water extract 30% shows the maximum microbial activity.HPLC analysis of fruits component, we observe orange methanolic extract and apricot methanolic extract shows the highest peak of components ***Tangerarin (3.35)*** *and* ***Resveratrol (12.54)*** *respectively with respect to retension time.The present study was carried out to evaluate the antihelmintic activity of fruit extract of orange and apricot using Indian earth warms. All the extracts were found not only the paralysis (vermifuge) but also to kill the earth warms (vermicidal).*

Keywords: Apricot and Orange peel, Antimicrobial activity, Antiheminthic activity, Enterococcus, HPLC, Identification of bacteria, Staphylococcus.

I. INTERODUCTION

16S rRNA gene is used for phylogenetic studies (W G Weisburg et al., 1991) as it is highly conserved between different species of bacteria and archaea (Coenye T, Vandamme P 2003). Sequence analysis of the 16S rRNA sequences are done with the help of several primers, called "universal primers." These primers target the conserved region of 16S rRNA gene and amplify the target in parts.

Finally the several amplified parts could be assembled together to have the entire sequence of the complete 16S rRNA. In addition to highly conserved primer binding sites, 16S rRNA gene sequences contain hypervariable regions that can provide species-specific signature sequences useful for bacterial identification. As a result, 16S rRNA gene sequencing has become prevalent in medical microbiology as a rapid, accurate alternative to phenotypic methods of bacterial identification (J. E. Clarridge III, 2004).Although it was originally used to

identify bacteria, 16S sequencing was subsequently found to be capable of reclassifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured (Weisburg WG 1991, Brett P J et al., 1998, Gray JP, Herwig RP 1996).

- **Staphylococcus**

Staphylococcus was first identified in Aberdeen, Scotland (1880) by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. This name was later appended to ***Staphylococcus aureus*** by Rosenbach who was credited by the official system of nomenclature at the time. It is estimated that 20% of the human population are long-term carriers of *S. aureus* which can be found as part of the normal skin flora and in anterior nares of the nasal passages. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection.

S. aureus is the most common species of staphylococcus to cause *Staph* infections and is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies. *Staphylococcus aureus* is a common bacterium found on the skin and in the noses of up to 25% of healthy people and animals. *Staphylococcus aureus* is important because it has the ability to make seven different toxins that are frequently responsible for food poisoning.

Staphylococcus aureus is a bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning.

- **Enterococcus**

Bacteria normally found in the feces. Two types, Enterococcus fecalis and Enterococcus fecium, cause human disease, most commonly in the form of urinary tract and wound infections. Other infections, including those of the blood stream (bacteremia), heart valves (endocarditis), and the brain (meningitis) can occur in severely ill patients in hospitals. Enterococci also often colonize open wounds and skin ulcers, and are among the most common antibiotic-resistant bacteria.*Enterococcus* spp. serve as a key reservoir of antibiotic resistance genes, and they disseminate these genes to other species. Enterococci have transferred resistance to the last line antibiotic, vancomycin, to methicillin-resistant *Staphylococcus aureus*, creating a pan-resistant 'superbug'. Enterococci themselves have emerged as leading causes of multiple antibiotic resistant hospital-acquired infection. Despite this medical importance, few enterococcal isolates have been sequenced with an accessible, annotated database. This project examines the organization of antibiotic resistance genes in enterococcal genomes, as well as those genes that may distinguish infection-related isolates from commensal isolates.Members of the genus *Enterococcus* were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated a separate genus classification would be appropriate.

Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis.Sensitive strains of these bacteria can be treated with ampicillin, penicillin and vancomycin. From a medical standpoint, an important feature of this genus is the high level of intrinsic antibiotic resistance. Some enterococci are intrinsically resistant to β-lactam-based antibiotics (penicillins,cephalosporins,carbapenems), as well as many aminoglycosides. In the last two decades, particularly virulent strains of *Enterococcus* that are resistant to vancomycin (vancomycin-resistant *Enterococcus*, or VRE)

have emerged in nosocomial infections of hospitalized patients, especially in the US. Other developed countries, such as the UK, have been spared this epidemic, and, in 2005, Singapore managed to halt an epidemic of VRE. VRE may be treated with quinupristin/dalfopristin (Synercid) with response rates of approximately 70%. Tigecycline has also been shown to have anti-enterococcal activity as has rifampicin.

II. MATERIAL AND METHOD

Isolation of Bacteria from Soil samples, To Perform Streak-Plate Technique, Preparation of Slants of Isolated Cultures, Staining Techniques, To perform gram staining of isolated bacteria(Pelczar/Chan/krieg Fifth edition, K.R. Aneja Fourth edition)

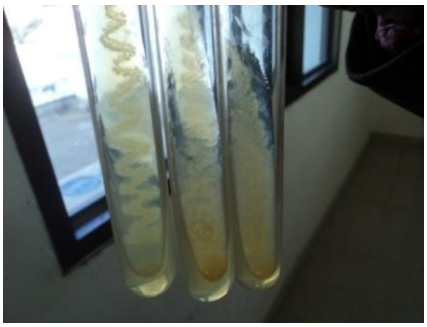
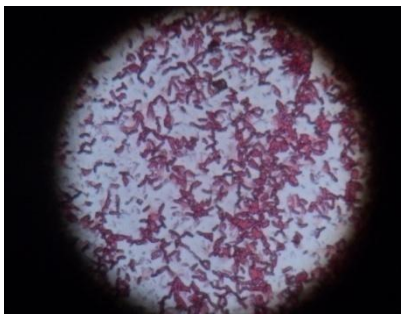
2.1 Biochemical Tests of Isolated Bacteria

Amylase production test (or starch hydrolysis), Sugar mannitol fermentation by bacteria Hydrolysis of gelatine, a protein (production of gelatinase), To perform urease test, Catalase activity, Methyl red test, To perform H_2S test, Voges Proskauer Test, Citrate Test, 6.5% NaCl Tolerance Test **Bergey's Manual:** Systematic Bacteriology is the main resource for determining the identity of bacteria species, utilizing every characterizing aspect. Identification flow charts of Bergey's Manual of Determinative Bacteriology is given in appendix.

2.2 Observations and Results Isolation

Different types of colonies were appeared on higher dilutions at 10^{-5}.Two different colour and shaped colonies were picked and maintained on Nutrient agar plates and slants for further experiments.

Streaking **Slants Culture of Isolated Bacteria** **Gram's staining**

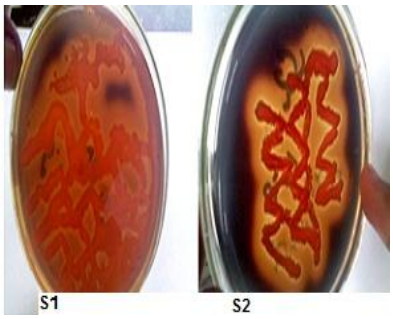
Gram's staining

	Sample 1	Sample 2
	-ve	-ve

International Journal of Advance Research in Science and Engineering
Vol. No.5, Issue No. 03, March 2016
www.ijarse.com
IJARSE
ISSN 2319 - 8354

Biochemical Tests

Amylase Production Test



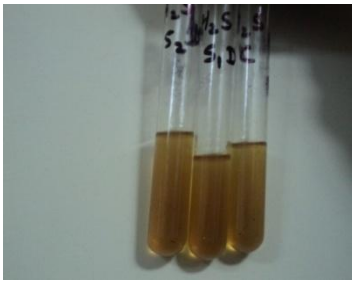
Hydrolysis Of Gelatin

Mannitol Fermentation

Urease Test

Catalase Activity

H_2S Test



Results

Light Microscopic view of gram stain results

Results of Biochemical Tests-

Tests	Sample 1	Sample 2
VP Test	+ve	+ve
MR Test	+ve	+ve
Catalase Test	+ve	+ve
Urease Test	+ve	+ve
Mannitol Test	-ve	-ve
Citrate Test	+ve	-ve
Starch Utilization Test	-ve	-ve
6.5% NaCl Tolerance Test	+ve	-ve

International Journal of Advance Research in Science and Engineering
Vol. No.5, Issue No. 03, March 2016
www.ijarse.com
IJARSE
ISSN 2319 - 8354

III. RESULTS OF 16S RDNA SEQUENCING METHOD

Both the techniques i.e. Biochemical tests and 16 S rDNA sequencing method were used to obtain results. Both the techniques showed similar results.

- **Sequencing results**

We obtained sequencing report from BIOSERVE biotechnology (india) Pvt Ltd. Hyderabad-500076

1-

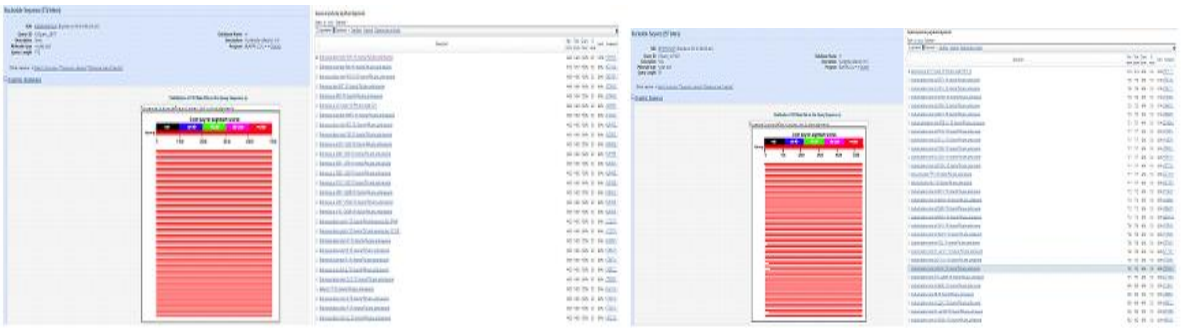
AGTAACACGTGGGTAACCTGCCCATCAGAAGGGGAGAACACTTGGAAACAGGTGCTAATACCGTATAACA
ATCGAAACCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATT
AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACA
TTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGT
CTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAA
GGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGC
CGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTA
AGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGA
GGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAAGCGGC
TCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
CACGCCGTAACGATGAGTGCTAGGTGTTGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCA
CT

2-

GATTGAGACAGGGCCCAGTTCGTATGTGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCTGAAT
GCAGCGAGCCTTCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGCAAGT
GACGGTACCTGCAGAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCG
TTGTCCGGAATTATTGGGCGTAAAGAGTTCGTAGGCGGTTTGTTGAGTCGTTTGTGAAAACCAGCAGGCT
CAACCGTGAGGGCCGTTGGATACGGGGAAAACTTGAGTACTGCAGGGGAGAGTGGAATTCCATGTGTAGC
GGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGA
TGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA
GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCNGGGANNNGNNC

Blast

S1 S2



IV. ANTIMICROBIAL

It was found that the extract of orange peel possessed maximum antimicrobial activity against *staphylococcus* in the ethanolic extract the higher concentration (30%).

Antimicrobial activity of Orange peel (mm)

Micro-organism	Days	Water conc.			Ethanol conc.			Methanol conc.		
Staphylococcus		10	20	30	10	20	**30**	10	20	30
	1	NA	8	8	NA	8	**8**	NA	NA	NA
	2	8	8	8	8	8	**9**	NA	NA	NA
	3	8	8	8	9	9	**9**	8	8	NA
	4	8	8	8	9	9	**9**	8	8	8
	5	9	9	9	9	9	**9**	8	8	8
	6	9	9	9	10	9	**10**	8	8	8
	7	9	10	9	11	9	**11**	10	9	9
	8	9	10	10	11	10	**11**	10	9	9
	9	10	11	11	11	10	**11**	10	9	9
	10	10	11	11	11	10	**11**	10	9	9

It was found that the extract of apricot peel possessed maximum antimicrobial activity against *staphylococcus* in the methanolic extract the lower concentration (10%).

Antimicrobial activity of Apricot peel (mm)

Micro-organism	Days	Water conc.			Ethanol conc.			Methanol conc.		
Staphylococcus		10	20	30	10	20	30	**10**	20	30
	1	8	8	8	8	8	NA	**8**	NA	8
	2	8	8	8	8	8	8	**8**	NA	8
	3	10	10	10	8	8	8	**9**	8	9
	4	10	10	10	9	9	9	**9**	8	9
	5	10	10	10	10	10	9	**9**	8	9
	6	11	11	10	10	10	10	**10**	9	10
	7	11	11	10	10	10	10	**11**	9	10
	8	11	12	12	10	10	10	**11**	10	10
	9	12	12	13	10	10	10	**11**	10	10
	10	13	12	13	10	11	11	**11**	11	11

It was found that the extract of orange peel possessed maximum antimicrobial activity against *Eterococcus* in the methanolic extract the higher concentration (30%).

Antimicrobial activity of Orange peel (mm)

Micro-organism	Days	Water conc.			Ethanol conc.			Methanol conc.		
Enterococcus		10	20	30	10	20	30	10	20	**30**
	1	8	NA	8	NA	8	8	NA	NA	**NA**
	2	8	8	8	NA	8	8	8	NA	**NA**
	3	8	8	8	8	8	8	8	8	**8**
	4	9	8	9	8	8	8	8	8	**8**
	5	9	9	11	8	8	8	8	8	**8**
	6	11	12	11	10	9	10	8	8	**14**
	7	11	12	12	11	10	10	12	11	**14**
	8	11	12	12	11	11	10	12	11	**14**
	9	11	12	12	11	11	10	12	11	**14**
	10	12	13	13	12	12	11	13	12	**15**

It was found that the extract of apricot peel possessed maximum antimicrobial activity against *Enterococcus* in the aqueous extract the higher concentration (30%).

Antimicrobial activity of Apricot peel (mm)

Micro-organism	Days	Water conc.			Ethanol conc.			Methanol conc.		
Enterococcus		10	20	**30**	10	20	30	10	20	30
	1	8	8	**8**	8	8	NA	NA	8	NA
	2	9	9	**9**	9	8	8	NA	8	NA
	3	12	10	**12**	9	8	8	8	9	8
	4	12	13	**12**	9	9	9	8	9	8
	5	12	13	**13**	10	11	10	8	9	8
	6	12	13	**13**	11	12	11	9	9	9
	7	12	13	**14**	11	12	12	9	10	10
	8	13	13	**14**	11	12	12	9	10	10
	9	14	13	**14**	11	12	12	9	10	10
	10	15	14	**15**	12	13	13	10	11	12

International Journal of Advance Research in Science and Engineering
Vol. No.5, Issue No. 03, March 2016
www.ijarse.com
IJARSE
ISSN 2319 - 8354

V. ANTIMICROBIAL ACTIVITY TEST RESULT

5.1 nthelmintic activity of Orange peel

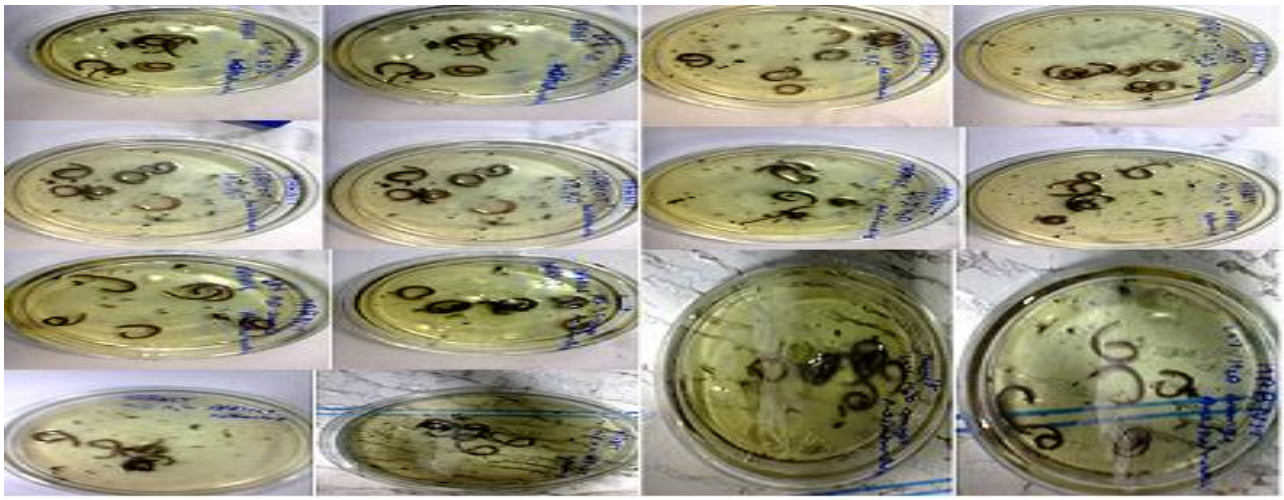
Group	Concentration (mg/ml)	Time(min)	
		Paralysis	**Death**
	10	-	-
Control standard (water)	20	-	-
	30	-	-
	10	2.23±0.10	4.45±0.35
Ethanolic extract	20	2.00±0.21	3.15±0.27
	30	**1.30±0.17**	**2.30±0.13**
	10	2.50±0.25	6.27±0.10
Metanolic extract	20	2.10±0.15	4.07±0.19
	30	**1.25±0.28**	**2.43±0.21**

5.2 Antihelminthic activity of Apricot peel

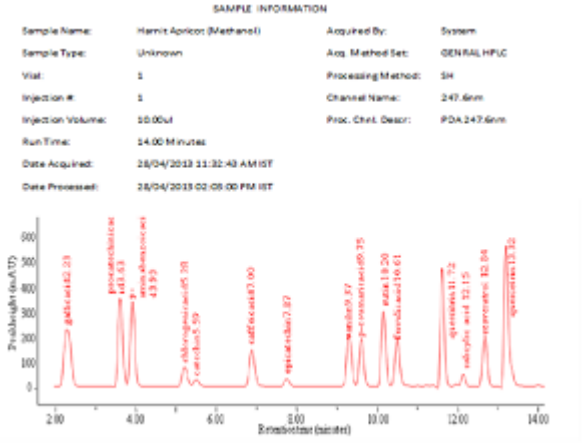
Group	Concentration (mg/ml)	Time(min)	
		Paralysis	**Death**
	10	-	-
Control standard (water)	20	-	-
	30	-	-
	10	4.29±0.39	8.08±0.27
Ethanolic extract	20	3.18±0.47	6.38±0.34
	30	**1.57±0.09**	**4.15±0.59**
	10	6.27±0.22	9.25±0.13
Metanolic extract	20	5.32±0.47	6.21±0.26
	30	**3.23±0.32**	**4.11±0.40**

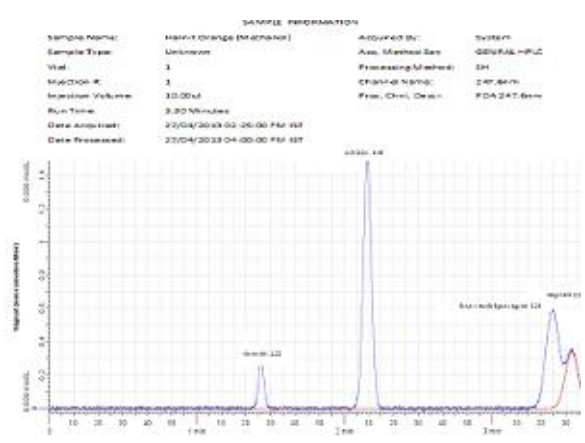
VI. ANTIHELMINTHIC ACTIVITY OF ETHNOLIC, METHANOLIC AND AQUEOUS EXTRACT

All concentration showed significant anthelmintic activity at all tested doses when compred to reference standard(Table) as vermifuge and vermicide. But 40mg/ml concentration shows more action than others.
Potency of the extract was inversely proportionl to time for analysis and death of worms.



HPLC analysis –HPLC chromatogram of standard apricot methenolic extract shows the peak of 12.84 (resveratrol) with respect to retension time . HPLC chromatogram of standard orange methenolic extract shows the peak of 2.35 (tangerarin) with respect to retension time .





VII. CONCLUSION

After analyzing two unknown bacteria samples by using both Biochemical tests and 16s rDNA technique, we came to the conclusion that the bacteria samples which I was analyzing, found to be ***Enterococcus and Staphylococcus***.Molecular identification of bacteria using 16S rDNA sequencing provides three primary advantages over phenotypic identification: rapid turn-around time, improved accuracy, and taxonomical

meaningfulness (Harmsen 2002). This study provides the molecular basis for accurate species identification.After that we used both bacteria for antimicrobial activity.

REFERENCES

[1] Tao K, 2007. Chemical composition of essential oil from the peel Satsuma mandarin. African journal of Biotechnology, 7(a):1261-1264.

[2] Caccioni DRL et al., 1998. Relationship between volatile components of citrus essential oils and antimicrobial action on Penicillium digitatum and penicillium italicum. Int. J. Food Microbiology, 43:73-79.

[3] Ahmed A, 2006. Genetic variability to essential oil composition in four Citrus fruit species. Pak. J. Bot., 38(2):319-324.

[4] Alade PI, Irobi ON, 1993. Antimicrobial activity of crude leaf extract of Acalypha wilkesiana. J. Ethanopharmacol.,39:170-174.

[5] Perez C et al., 1990. An antibiotic assay by the agar well diffusion method. Acta. Bio. Med. Exp., 15:113-115.

[6] Ruch RJ, Cheng SJ, Klaunig JE, 1989. Carcinogenesis, 10:1003-1008.

[7] Kumar Ashok B.S., Lakshman K., Jayaveera K.N., Nandeesh R., Manoj B. and Ranganayakulu D, 2010. "Comperative in vitro anthelmintic activity of three plants of Amaranthaceae family" Arch Biol Sci. 62 (1): p.185-89.

[8] Lateef M., Iqbal Z., Khan M.N., Aktar M.S. and Jabbar A, 2003. "Anthelmintic activity of Adhatoda vasica roots" Int J Agri Biol. 5 (1): p. 86-90.

[9] Deore S.L. and Khadabadi S.S., 2010. "In vitro anthelmintic studies of Chlorophytum borivilianum Sant. & Fernandez tubers" Indian J Nat Prod Resources 1 (1): p. 53-56.

[10] Aswar M., Aswar U., Watkar B., Vyas M., Wagh A. and Gujar KN, 2008. "Anthelmintic activity of Ficus benghalensis" International Journal of Green Pharmacy 2 (3): p. 170-172.

[11] Mendhe BB., Nema U., Gupta P. and Gandhare BR, 2010. "Evaluation of Anthelmintic activity of leaf extracts of Butea Monosperma" International Journal of Pharmaceutical Sciences and Research 1(3): p. 69-72.

[12] Qureshi S. Md., Patel J., Giri C. I., Hasan S., Shaik M. and Hamed Md, 2010. "In vitro anthelmintic activity of root extract of Trapa bispiniosa Roxb. Against Pheretima posthuma and Ascardia gilli. Study of anthelmintic activity" International Journal of Pharma Science and Research 1 (9): p. 353-356.

[13] Dey P., Debnath P. and Bhakta T, 2012. "Evaluation Of Anthelmintic Activity Of Molineria Recurvata Leaf Extracts" Int. Res J Pharm. App Sci. 2 (2): p. 16-20.

[14] Karale S.S., Godad A.P., Yashodhan B.W. and Suhas A.S, 2010. "Evaluation of in vitro anthelmintic activity of Ceratophyllum demersum Linn." Indian Drugs 47 (8): p. 63-64

[15] Bate-Smith E.C. (1962) J Soc Bot, 58: 95-103.