AMERICAN JOURNAL OF MANAGEMENT AND IOT MEDICAL COMPUTING
PEER REVIEWED, REFERRED & INDEXED JOURNAL
E-ISSN: 3069-0110
Vol.3, No. 3(2024)
www.ajmimc.com

INVITRO ASSESSMENT OF ANTIMICROBIAL AND ANTIHELMINTHIC ACTIVITY OF PEACH AND ORANGE PEEL BY BACTERIA ISOLATED FROM PIGEON DROPPING CLOSE TO HOSPITAL

Deepak Chand [1], Antra Manjhi [2]
Smt. Vidyawati College of Education, Jhansi
Shri Rawatpura Sarkar College of Pharmacy, Datia

ABSTRACT
The peach (*Prunus persica*), a deciduous tree from Northwest China, produces a sweet, nutrient-rich fruit known for its velvety skin. Peaches are low in calories and packed with vitamins A and C, fiber, potassium, and antioxidants, making them a healthy dietary choice. On the other hand, orange peel, often discarded as waste, is rich in bioactive compounds like essential oils and flavonoids, valuable for culinary, medicinal, and cosmetic uses. This study evaluated the antimicrobial and anthelmintic properties of aqueous, ethanolic, and methanolic extracts from fruit peels. The orange ethanolic extract exhibited the strongest antimicrobial activity against *Staphylococcus aureus*, while the apricot methanolic extract was most effective at 10% concentration against the same bacteria. The orange methanolic extract at 30% concentration showed significant activity against *Enterococcus*. HPLC analysis revealed Tangeretin and Resveratrol as key components. Additionally, all extracts demonstrated effective antihelmintic activity, causing paralysis and death in Indian earthworms.

Keywords: Peach and Orange peel, Antimicrobial activity, Antihelminthic activity, Enterococcus, HPLC, Identification of bacteria, Staphylococcus.

INTRODUCTION

The 16S rRNA gene is widely used in phylogenetic studies due to its high level of conservation among various bacterial and archaeal species (Weisburg et al., 1991; Coenye & Vandamme, 2003). Sequence analysis of 16S rRNA involves the use of "universal primers," which are designed to target conserved regions of the gene, allowing for the amplification of specific segments. These amplified segments can then be assembled to reconstruct the entire 16S rRNA gene sequence. In addition to the conserved regions, the 16S rRNA gene also contains hypervariable regions that provide species-specific signature sequences, making it a valuable tool for bacterial identification. Consequently, 16S rRNA gene sequencing has gained widespread use in medical microbiology as a rapid and accurate alternative to traditional phenotypic methods for identifying bacteria (Clarridge III, 2004). Initially employed for bacterial identification, 16S sequencing has also enabled the reclassification of bacteria into new species or even new genera, and it has been instrumental in identifying new species that have not been successfully cultured (Weisburg et al., 1991; Brett et al., 1998; Gray & Herwig, 1996).

Staphylococcus was first discovered in Aberdeen, Scotland, in 1880 by surgeon Sir Alexander Ogston, who found it in pus from a knee joint abscess. The name was later attached to Staphylococcus aureus by Rosenbach, who was recognized by the nomenclature system of the time. Around 20% of people are long-term carriers of S. aureus, which is normally found on the skin and in the nasal passages. Each year, about 500,000 patients in American hospitals develop staphylococcal infections. S. aureus is the primary species responsible for Staph infections and is a successful pathogen due to its ability to persist in the nose and evade the immune system. It is commonly found on the skin and in the noses of up to 25% of healthy individuals and animals. S. aureus is significant because it can produce seven different toxins that often cause food poisoning. As a member of the Firmicutes, S. aureus is usually present in the human

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

respiratory tract and on the skin. Although not always pathogenic, it frequently causes skin infections (like boils), respiratory issues (such as sinusitis), and food poisoning.

Bacteria commonly found in feces include Enterococcus faecalis and Enterococcus faecium, which can cause infections in humans, particularly urinary tract and wound infections. In severely ill hospital patients, they may also lead to infections of the bloodstream (bacteremia), heart valves (endocarditis), and brain (meningitis). Enterococci frequently colonize open wounds and skin ulcers and are notable for their high level of antibiotic resistance. They are a major source of antibiotic resistance genes and can transfer these genes to other bacteria. For example, they have passed resistance to the last-resort antibiotic vancomycin to methicillin-resistant Staphylococcus aureus, creating a pan-resistant 'superbug'. Enterococci are now significant causes of multi-drug-resistant hospital-acquired infections. Despite their medical importance, few enterococcal strains have been fully sequenced with a comprehensive database. This project aims to investigate the organization of antibiotic resistance genes in enterococcal genomes and identify genes that may differentiate infection-related strains from those that are merely part of the normal flora. Previously classified as Group D Streptococcus until 1984, enterococci were reclassified into their own genus based on genomic DNA analysis. Clinically significant infections caused by Enterococcus include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. Sensitive strains can be treated with ampicillin, penicillin, and vancomycin. However, enterococci exhibit high intrinsic antibiotic resistance, being naturally resistant to β-lactam antibiotics (such as penicillins, cephalosporins, and carbapenems) and many aminoglycosides. In recent decades, particularly virulent strains resistant to vancomycin (vancomycin-resistant Enterococcus, or VRE) have emerged. Vancomycin-resistant Enterococcus (VRE) has become a significant issue in hospital-acquired infections, particularly in the US. However, other developed countries, such as the UK, have largely avoided this epidemic. In 2005, Singapore successfully contained an outbreak of VRE. Treatment options for VRE include quinupristin/dalfopristin (Synercid), which has a response rate of about 70%. Additionally, tigecycline and rifampicin have also been found to be effective against enterococcal infections.

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

Biochemical Tests of Isolated Bacteria

Amylase production test (or starch hdrolysis), Sugar mannitol fermentation by bacteria Hydrolysis of gelatine, a protein (production of gelatinase), To perform urease test, Catalase activity, Methyl red test, To perform H2S 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 chart of Bergey's Manual of Determinative Bacteriology is given in appendix.

Observations and Results Isolation

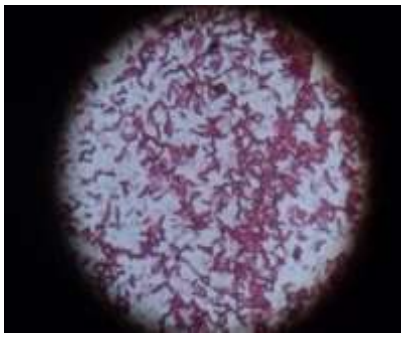
Different types of colonies were appeared on higher dilutions at 10^{-5}.Two different colour and shaped colonies were picked and maintained on Nutrient agar plates and slants for further experiments.

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

AMERICAN JOURNAL OF MANAGEMENT AND IOT MEDICAL COMPUTING
PEER REVIEWED, REFERRED & INDEXED JOURNAL
E-ISSN: 3069-0110
Vol.3, No. 3(2024)
www.ajmimc.com

Streaking

Slants Culture of Isolated Bacteria



Gram's staining

Gram's staining

Sample 1	Sample 2
-ve	-ve

Biochemical tests

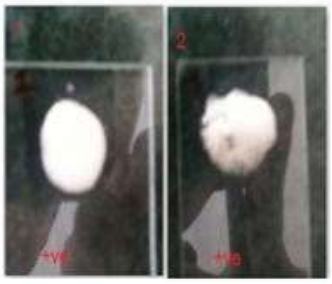
Amylase Production Test

Hydrolysis Of Gelatin

Mannitol Fermentation

Urease Test

Catalase Activity



H_2S Test

Results

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

AMERICAN JOURNAL OF MANAGEMENT AND IOT MEDICAL COMPUTING
PEER REVIEWED, REFERRED & INDEXED JOURNAL
E-ISSN: 3069-0110
Vol.3, No. 3(2024)
www.ajmimc.com

Light Microscopic view of gram stain results

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

RESULTS OF 16S RDNA SEQUENCING METHOD

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

- **Sequencing results**

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

  1-

  AGTAACACGTGGGTAACCTGCCCATCAGAAGGGGAGAACACTTGGAAACAGGTGC
  TAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGT
  GTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACC
  AAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGA
  CACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAA
  AGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTG
  TTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACC
  AGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
  GTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGT
  GAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCA
  GAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGA
  ACACCAGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCG
  TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTGCTA
  GGTGTTGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCACT

  2-

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

GATTGAGACAGGGCCCAGTTCGTATGTGAGGCAGCAGTGGGGAATATTGCACAATG
GGCGAAAGCTGAATGCAGCGAGCCTTCGTGAGGGATGACGGCCTTCGGGTTGTAAA
CCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCCCCGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTGTCCGGAATTAT
TGGGCGTAAAGAGTTCGTAGGCGGTTTGTTGAGTCGTTTGTGAAAACCAGCAGGCT
CAACCGTGAGGGCCGTTGGATACGGGGAAAACTTGAGTACTGCAGGGGAGAGTGG
AATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAA
GGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGT
TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCNGGGANNNGNNC

ANTIMICROBIAL

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

Antimicrobial activity of Peach 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

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

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* inthe 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**

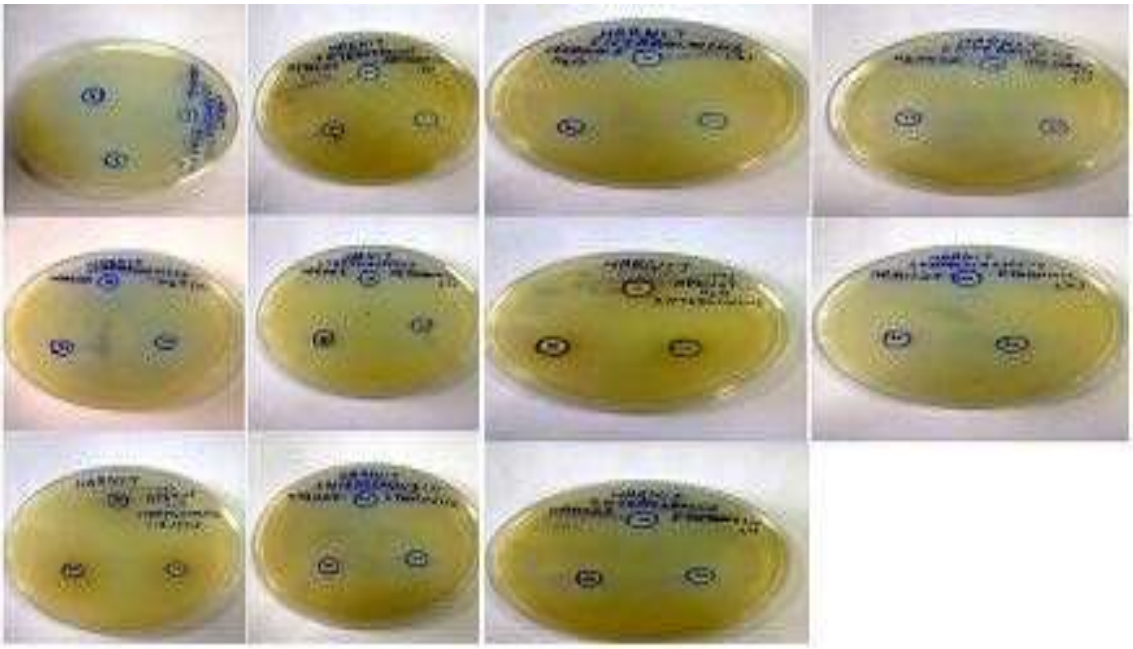
It was found that the extract of peach peel possessed maximum antimicrobial activity against *Enterococcus* inthe aqueous extract the higher concentration (30%).

Antimicrobial activity of Peach t 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

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

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



ANTIMICROBIAL ACTIVITY TEST RESULT

Antihelmintic activity of Orange peel

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

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

AMERICAN JOURNAL OF MANAGEMENT AND IOT MEDICAL COMPUTING
PEER REVIEWED, REFERRED & INDEXED JOURNAL
E-ISSN: 3069-0110
Vol.3, No. 3(2024)
www.ajmimc.com

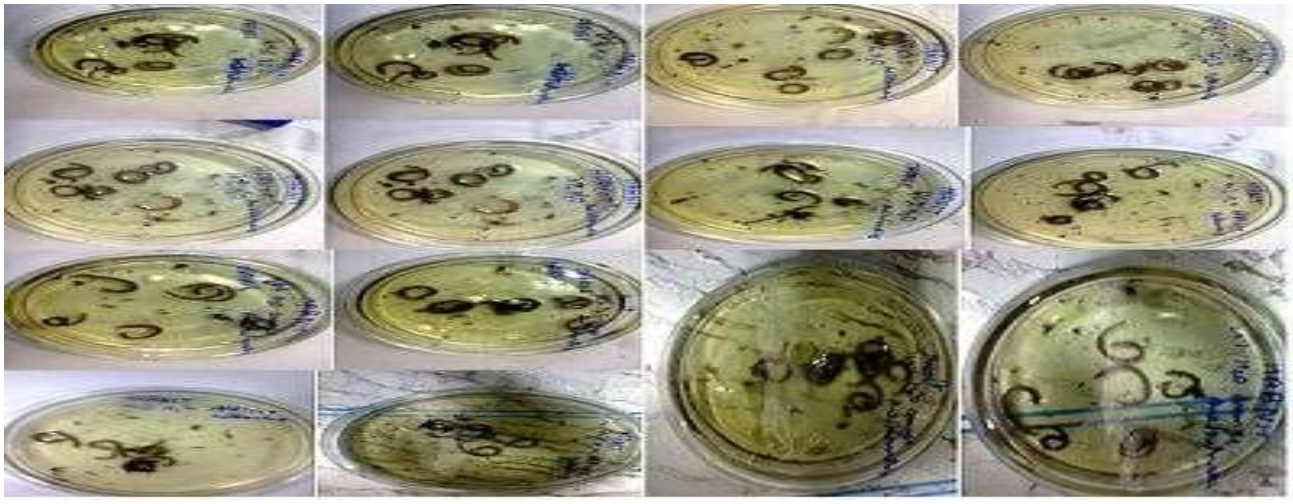
Antihelminthic activity of Peach peel

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

ANTIHELMINTHIC ACTIVITY OF ETHNOLIC, METHANOLIC AND AQUEOUS EXTRACT

All concentration showed significant anthelmintic activity at all tested doses when compared to reference standard (Table) as vermifuge and vermicide. But 40mg/ml concentration shows more action than others.

Potency of the extract was inversely proportional 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 retention time. HPLC chromatogram of standard orange methenolic extract shows the peak of 2.35 (tangerarin) with respect to retention time.

CONCLUSION

After analyzing two unknown bacteria samples by using both Biochemical tests and 16s rDNA

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |

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.

REFERENCES

1. Abulreesh, H.H., Organji, S.R., Elbanna, K., Osman, G.E.H., Almalki, M.H.K., Abdel-Malek, A.Y., Ghyathuddin, A.A.K., Ahmad, I. (2019). Diversity,Virulence Factors, and Antifungul Susceptibility Patterns of Pathogenic and Opportunistic Yeast Species in Rock Pigeon (Columba livia) Fecal Droppings in Western Saudi Arabia. Polish Journal of Microbiology,68, 493-504.
2. Bhumbla U., Majumdar S., Jain S., Dalal AS. (2020) A study of isolation and identification of bacteria from lake water in and around Udaipur, Rajasthan. Journal of Family Medicine and Primary Care 2020; 9:751-4.
3. Brown A.E. in: P.E. Reidy, J.S. Fornango (Eds.), (2005). Benson's Microbiological Applications: Laboratory Manual in General Microbiology, McGraw-Hill, Inc, New York
4. Perez C et al., 1990. An antibiotic assay by the agar well diffusion method. Acta. Bio. Med. Exp., 15:113-115.
5. 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.
6. Charan Nandigama, N. (2024). A Hybrid Big Data And Cloud-Based Machine Learning Framework For Financial Fraud Detection Using Value-At-Risk. International Journal of Research and Analytical Reviews, 11(3). https://doi.org/10.56975/ijrar.v11i3.324899
7. 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.
8. Charan Nandigama, N. (2020). An Integrated Data Engineering and Data Science Architecture for Scalable Analytical Warehousing and Real-Time Decision Systems. International Journal of Business Analytics and Research (IJBAR).
9. 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.
10. 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.
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. 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.
13. 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.
14. 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.

Received: 06-07-2024 | Accepted: 10-08-2024 | Published: 19-08-2024 |