OPEN ACCESS

Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

In vitro Bioactivity of Halophytic Plant *Heliotropium curassavicum* L. against Selected Pathogens

K. Prasanna Lakshmi[1], G. M. Narasimha Rao[2*]

[1]Assistant Professor in Botany, M. R. College (A), Vizianagaram-535002, Andhra Pradesh, India
[2]Department of Botany, Andhra University, Visakapatnam, Andhra Pradesh, India

DOI: 10.36348/sjls.2023.v08i10.002 | **Received:** 04.10.2023 | **Accepted:** 10.11.2023 | **Published:** 14.11.2023

***Corresponding author:** G. M. Narasimha Rao
Department of Botany, Andhra University, Visakapatnam, Andhra Pradesh, India

Abstract

Heliotropium curassavicum L. was screened for antimicrobial activity against some plant and human pathogens. Plant parts of *H. curassavicum*, were collected from mangrove habitats of Chollangi, near Kakinada, Plant parts are dried and extracts were obtained successfully with hexane, chloroform, methanol and water, by using Soxhlet extraction apparatus. The antimicrobial activity of the plant extracts on the various test organisms, including multiple antibiotic resistant bacteria were investigated. Antimicrobial activity of the extracts was determined by the Well Diffusion Method. The results concluded that the leaf and stem extracts of *H. curassavicum* possess antibacterial, antifungal activities. There is a possibility of developing this plant as a source of antibacterial and antifungal agent and further investigations are necessary to identify the bioactive principles.

Keywords: Halophytes, Antimicrobial Activity, *In vitro* Bioactivity, Multiple Antibiotic Resistant Bacteria, Well Diffusion Method, *Heliotropium curassavicum*.

INTRODUCTION

Halophytes have long been used for medicinal purposes (Ferreira *et al.*, 2022). They are traditionally used for medicines and the release of bioactive compounds (Arya *et al*., 2019). Several species of halophytes are used as folk medicine and prove that halophytes have anti-pathogenic activity. Antimicrobial activity is associated with secondary metabolites, such as phenolic acids, flavonoids and tannins. Halophytes possess alkaloids, phenols, steroids, terpenoids, tannins, etc. (Combs and Anderson, 1949; Patra and Thatoi, 2011; Falleh *et al.*, 2011; Qadir *et al.*, 2017). Antimicrobial properties of different halophytes of Godavari estuary were studied by Prasanna Lakshmi and Narasimha Rao, (2013); Prasanna Lakshmi *et al.*, (2015); Prasanna Lakshmi and Narasimha Rao, (2023a); Prasanna Lakshmi and Narasimha Rao, (2023b).

Heliotropium belongs to family Boraginaceae, is used in Nigeria to treat ailments such as ulcer and fever (Adelaja *et al*., 2008). Phytochemical reports on genus *Heliotropium* revealed the presence of many bioactive components especially pyrrolizidine alkaloids, terpenoids, flavonoids and saponins (Reina *et al.*, 1997; Hussain *et al*., 2010; Osungunna and Adedeji, 2011; Ghori *et al.*, 2016). Species of *Heliotropium* produce resinous exudates that cover the leaves and stems. These exudates are associated with a complex defence mechanism that includes the prevention of phytopathogens, and phytophagous organisms. The plant distributed throughout the east and west coast of India including Godavari estuary (Chollangi), Kakinada, Andhra Pradesh (Umamaheswara Rao and Narasimha Rao, 1988; Narasimha Rao, 2012 and Prasanna Lakshmi, 2015).

The present study we have attempted to evaluate the antimicrobial activity of halophytic plant *H. curassavicum* against some plant and human pathogens.

MATERIALS AND METHODS

Plant Material:

Plant materials of *H. curassavicum* (Fig 1) were collected during 2010 to 2011 from mangrove habitats of Chollangi near Kakinada, Andhra Pradesh, India. Plant materials were identified with the help of authentic specimens available in the Department of Botany, Andhra University, and Visakhapatnam.

Fig 1: *Heliotropium curassavicum* L.

Test Microorganisms:

The selected bacterial strains were obtained from Microbial Type Culture and collection (MTCC) from Institute of Microbial Technology, Chandigarh, India. The microorganisms including bacteria and fungi were presented in the Table-1 and Table-2 respectively.

Table-1: Details of the Bacterial strains used in Bioassay

S. No	Name of the Bacterial Strains	MTCC Catalogue No.
1.	*Bacillus subtilis*	B 2274
2.	*Bacillus megaterium*	B 2444
3.	*Lactobacillus acidophilus*	B 5463
4.	*Escherichia coli*	B 9637
5.	*Enterobacter aerogenes*	B 2822
6.	*Enterobacter cloacae*	B 7982
7.	*Klebsiella pneumonia*	B 2405

Table-2: Details of the Fungal stains used in Bioassay

S. No	Name of the Fungal Strains	MTCC Catalogue No.
1.	*Candida albicans*	F 0227
2.	*Mucar racemosus*	F 7382
3.	*Rhizoctonia solani*	F 5642
4.	*Rhizopus stolonifer*	F 2591
5.	*Saccharomyces cerevisiae*	F 0174

Preparation of Plant Extracts:

The epiphytes and other deposits are removed from the halophytic plant and then the specimens are shade dried. The shade dried plant materials were chopped into small pieces and coarsely powdered. The coarsely powdered material was weighed and extracted with hexane, chloroform, methanol and water in sequential order of polarity using a soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2 ml of solvent was used. The extracted solvents were filtered though Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacuo at 40^0C) using a rotary evaporator. The residue obtained was designated as crude extract and was stored in a freezer at -20^0 C until bioassayed. The plant extract residues obtained were redissolved in 0.1% Dimethyl Sulfoxide (DMSO) to get different concentrations (100 mg/ml, 300 mg/ml and 500 mg/ml) and filtration through a 0.45 µm membrane filter and stored in sterile brown bottles at 20^0 C until bioassayed.

***In vitro* Antibacterial Activity Assays:**

The antimicrobial activity of the hexane, chloroform, methanol and water extracts of each sample was evaluated by using Agar Well Diffusion Method of Murray *et al.,* (1995) modified by Olurinola, (1996). 20ml of sterilized agar medium (Nutrient Agar Media for bacteria and Potato Dextrose Agar or PDA for fungi) was dispensed into sterile universal bottles. These were then inoculated with 0.2 ml of bacterial cultures, media was mixed gently and poured into sterile petri dishes and it is allowed to solidify. Then the 4 uniform wells were made in each petri dish by using a sterilized number 3-cup borer (6mm diameter). The wells were filled with 50—µl of the extract concentration of 100mg/ml, 300mg/ml,

500mg/ml and control (DMSO) and allowed diffusion for 45 minutes. The plates were incubated at 37° C for 24 hours for bacteria and 25° C for 48 hours for fungi. The zones inhibition was measured with antibiotic Zone Scale in mm and the experiment was carried out in duplicates.

RESULTS & DISCUSSION

Hexane, chloroform, methanol and water extracts of *H. curassavicum* leaves and stem exhibited the different degree of growth inhibition against tested bacterial and fungal strains in the present study. The data (values of Inhibition Zones (IZ)) pertaining to the antimicrobial potential of the leaves and stem of four solvents such as hexane, chloroform, methanol and water (100 mg/ml, 300 mg/ml and 500 mg/ml) presented in tables 3 and 4 respectively. In the present investigation there was a gradual increase in the zone of inhibition from 100 to 500mg/ml, with highest at 500 mg/ml concentration of plant extract. Hence only 500 mg/ml dosage level results were analyzed. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive.

Antimicrobial Activity of *Heliotropium curassavicum* Leaves

Hexane Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the hexane extracts of *H. curassavicum* leaves, against bacterial strain such as *L. acidophilus* (21 mm). While moderate level of antimicrobial activity was found against bacterial strains such as *E. coli* (18.7 mm) followed by *E. aerogenes* ((18.3 mm) and *B. megaterium* (18.3 mm) whereas fungal strains such as *M. recemosus* (19.3 mm) followed by *C. albicans* (16.7 mm) and *S. cerevisiae* (16.3 mm) (Table-3).

Extracts of 500 mg/ml

Moderate level of antimicrobial activity was recorded for the chloroform extracts of *H. curassavicum* leaves, against bacterial strains such as *B. megaterium* (20.3 mm) followed by *E. cloacae* (19.7 mm) whereas moderate antimicrobial activity observed against fungal strains such as *S. cerevisiae* (18 mm) followed by *R. stolonifer* (16.7 mm). No activity was found with the bacterial strain of *K. pneumonia* whereas fungal strain *M. recemosus* resistant to the extract (Table-3).

Methanol Extracts of 500 mg/ml

Moderate level of antimicrobial activity was found with the methanol extracts of *H. curassavicum* leaves, against bacterial strains such as *B. subtilis* (17 mm) followed by *B. megaterium* (15.7 mm) whereas fungal strain such as *C. albicans* (16.3 mm). Less antimicrobial activity of bacterial strains observed against *L. acidophilus* (15.3 mm) followed by *E. coli* (15.6 mm) and *E. aerogenes* (15.3 mm) whereas fungal strains such as *R. solani* (15.6 mm) followed by *M. recemosus* (13.7 mm) (Table-3).

The results in the present study are in accordance with the antimicrobial activity of methanol extract of *H. indicum* studied by Osungunna and Adedeji, (2011) against bacterial strains such as *Staphylococcus aureus* and *Klebsiella* sp.

Water Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded the water extracts of *H. curassavicum* leaves, against bacterial strains such as *B. subtilis* (22.3 mm), followed by *L. acidophilus* (22.3 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *K. pneumonia* (20 mm) followed by *E. cloacae* (21.3 mm) whereas fungal strains such as *M. recemosus* (18 mm) followed by *R. stolonifer* (17.7 mm), *C. albicans* (17.7 mm) and *S. cerevisiae* (17 mm). Less antifungal activity observed against *R. solani* (14 mm) (Table-3).

Similarly Hussain *et al*., (2010) studied antimicrobial activity of crude extract of *H. stigosum*. The plant exhibited excellent antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, moderate activity against methicillin resistant *Staphylococcus aureus* and *Bacillus subtilis*.

Antimicrobial Activity of *Heliotropium curassavicum* Stem

Hexane Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the hexane extracts of *H. curassavicum* stem, against fungal strain such as *M. recemosus*. Moderate level of antimicrobial activity was found in fungal strains such as *C. albicans* (18 mm) followed by *S. cerevisiae* (17.7 mm), *R. solani* (17.3 mm) and *R. stolonifer* (17.3 mm). Absence of antibacterial activity observed in all bacterial strains (Table-4).

Chloroform Extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the chloroform extracts of *H. curassavicum* stem, against fungal strain such as *S. cerevisiae* (21.3 mm). Moderate level of antimicrobial activity was found in bacterial strains such as *B. subtilis* (18.7 mm) followed by *B. megaterium* (19 mm). Less antifungal activity observed against fungal strains such as *C. albicans* (15 mm) followed by *M. recemosus* (13.7 mm). Absence of antifungal activity observed in *R. solani* ((Table-4). The results in the present study are in accordance with Hussain *et al*., (2010) reported that the chloroform and n-hexane extracts were active against four fungal strains such as *Aspergillus niger*, *A. fumigatus*, *Fusarium solani* and *A. flavus*.

Methanol Extracts of 500 mg/ml

Highest level of antimicrobial activity was found with the methanol extracts of *H. curassavicum* stem, against fungal strains such as *M. recemosus* (24 mm) followed by *R. stolonifer* (21.3 mm) and *S. cerevisiae* (21 mm). Moderate level of antimicrobial

activity was found in bacterial strain such as *B. megaterium* (18.7 mm) followed by *L. acidophilus* (18.3 mm) and *E. aerogenes* (18.3 mm) whereas fungal strain such as *R. solani* (17.7 mm). Less antibacterial activity was found against *E. cloacae* (14.6 mm) (Table-4).

Water extracts of 500 mg/ml

Highest level of antimicrobial activity was recorded for the water extracts of *H. curassavicum* stem, against bacterial strain such as *B. subtilis* (21.3 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *B. megaterium* followed by *E. coli* (17.7 mm), *E. aerogenes* (17.7 mm) and *K. pneumonia* (17.7 mm) whereas fungal strains such as *S. cerevisiae* (19.3 mm) and *R. stolonifer* (16.6 mm). Less antibacterial activity found against *L. acidophilus* (15.3 mm) and *E. cloacae* (15.3 mm), whereas fungal strains such as *M. recemosus* (23.7 mm) followed by *R. solani* (15.7 mm), (Table-4).

Table-3: Antimicrobial activities of *Heliotropium curassavicum* leaf extract in four different solvents- Hexane, Chloroform, Methanol and Water

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Standard
	H	C	M	W	H	C	M	W	H	C	M	W	
Bacillus subtilis **GP**	**11.7 ±0.6**	**-**	**12.3 ±0.6**	**15.3 ±0.6**	**15.6 ±1.2**	**-**	**14.3 ±0.6**	**18.0 ±1.0**	**17.0 ±1.0**	**17.3 ±1.6**	**17.0 ±1.0**	**22.3 ±1.6**	**32**
Bacillus megaterium **GP**	**12.0 ±0.0**	**13.3 ±0.6**	**11.7 ±0.6**	**14.3 ±0.6**	**16.3 ±0.6**	**17.7 ±1.2**	**13.7 ±0.6**	**17.7 ±1.2**	**18.3 ±0.6**	**20.3 ±1.5**	**15.7 ±0.6**	**21.3 ±0.6**	**28**
Lactobacillus acidophilus **GP**	**13.7 ±1.2**	**-**	**11.7 ±1.2**	**12.7 ±1.5**	**14.6 ±0.6**	**-**	**13.3 ±1.5**	**16.7 ±1.2**	**21.0 ±1.0**	**17.7 ±0.6**	**15.3 ±0.6**	**22.3 ±0.6**	**30**
Escherichia coli **GN**	**13.3 ±1.5**	**12.7 ±1.5**	**10.6 ±0.6**	**13.3 ±1.2**	**15.7 ±0.6**	**15.6 ±1.2**	**13.7 ±1.2**	**15.7 ±0.6**	**18.7 ±0.6**	**17.0 ±1.0**	**15.6 ±1.2**	**19.3 ±0.6**	**34**
Enterobacter aerogenes **GN**	**12.3 ±0.6**	**-**	**-**	**13.7 ±1.2**	**15.3 ±1.2**	**-**	**12.7 ±1.5**	**15.3 ±1.2**	**18.3 ±1.2**	**14.6 ±0.6**	**15.3 ±0.6**	**21.3 ±1.5**	**31**
Enterobacter cloacae **GN**	**12.0 ±1.0**	**-**	**11.7 ±1.2**	**13.3 ±1.5**	**15.3 ±0.6**	**13.7 ±1.2**	**13.3 ±0.6**	**16.3 ±0.6**	**17.3 ±1.6**	**19.7 ±0.6**	**14.6 ±0.6**	**19.3 ±0.6**	**32**
Klebsiella pneumonia **GN**	**12.3 ±0.6**	**-**	**11.3 ±0.6**	**13.7 ±0.6**	**15.3 ±1.5**	**-**	**13.0 ±1.0**	**17.7 ±0.6**	**17.7 ±1.2**	**-**	**14.6 ±0.6**	**20.0 ±1.0**	**31**
Candida albicans **FS**	**-**	**-**	**-**	**12.0 ±1.0**	**14.6 ±0.6**	**13.7 ±0.6**	**13.7 ±0.6**	**13.7 ±1.2**	**16.7 ±0.6**	**15.7 ±0.6**	**16.3 ±0.6**	**17.7 ±1.2**	**35**
Mucar recemosus **FS**	**-**	**-**	**13.0 ±1.0**	**10.6 ±0.6**	**16.7 ±1.2**	**13.3 ±1.5**	**15.3 ±0.6**	**15.6 ±1.2**	**19.3 ±0.6**	**-**	**13.7 ±0.6**	**18.0 ±1.0**	**32**
Rhizoctonia solani **FS**	**-**	**10.6 ±0.6**	**-**	**-**	**11.7 ±0.6**	**13.7 ±1.2**	**12.0 ±0.0**	**11.7 ±1.5**	**13.7 ±0.6**	**15.3 ±0.6**	**15.6 ±1.2**	**14.3 ±0.6**	**34**
Rhizopus stolonifer **FS**	**-**	**11.7 ±1.2**	**-**	**12.0 ±0.0**	**12.3 ±0.6**	**13.7 ±0.6**	**10.6 ±0.6**	**15.3 ±0.6**	**14.6 ±0.6**	**16.7 ±1.2**	**13.3 ±0.6**	**17.7 ±0.6**	**30**
Saccharomyces cerevisiae **FS**	**-**	**11.7 ±1.5**	**11.7 ±0.6**	**-**	**12.7 ±1.5**	**16.0 ±1.0**	**13.7 ±0.6**	**15.7 ±0.6**	**16.3 ±0.6**	**18.3 ±0.6**	**15.7 ±0.6**	**17.0 ±1.0**	**29**

Volume per well: 50µl; Borer size used: 6mm; **H**-Hexane, **C**-Chloroform, **M**-Methanol and **W**-Water.
GP = Gram positive; **GN** = Gram negative; **FS** = Fungal species and (-) indicates 'No inhibition'.
Diameter of zone of inhibition (mm) including disc diameter of 6mm: mean of three assays ± standard deviation.

Table-4: Antimicrobial activities of *Heliotropium curassavicum* stem extract in four different solvents- Hexane, Chloroform, Methanol and Water

Microorganisms	100mg/ml				300mg/ml				500 mg/ml				Standard
	H	C	M	W	H	C	M	W	H	C	M	W	
Bacillus subtilis **GP**	-	11.7 ±1.2	12.0 ±0.0	12.3 ±0.6	-	13.7 ±0.6	16.3 ±0.6	16.7 ±1.2	-	18.7 ±0.6	17.7 ±1.2	21.3 ±1.5	32
Bacillus megaterium **GP**	-	13.0 ±1.0	13.3 ±1.2	13.3 ±0.6	-	15.3 ±0.6	15.6 ±1.2	15.6 ±1.2	-	19.0 ±1.0	18.7 ±0.6	18.0 ±1.0	28
Lactobacillus acidophilus **GP**	-	12.3 ±0.6	10.6 ±0.6	-	-	16.3 ±0.6	14.6 ±0.6	13.3 ±1.2	-	17.7 ±0.6	18.3 ±0.6	15.3 ±0.6	30
Escherichia coli **GN**	-	11.7 ±1.2	13.3 ±1.2	12.7 ±1.2	-	14.6 ±0.6	15.3 ±1.2	15.7 ±0.6	-	16.7 ±1.2	16.7 ±1.2	17.7 ±0.6	34
Enterobacter aerogenes **GN**	-	-	11.0 ±1.0	13.3 ±0.6	-	14.3 ±0.6	15.3 ±1.2	15.6 ±1.2	-	17.0 ±1.0	18.3 ±0.6	17.7 ±0.6	31
Enterobacter cloacae **GN**	-	12.0 ±0.0	10.6 ±0.6	-	-	13.7 ±1.2	15.6 ±1.2	-	-	15.6 ±1.2	14.6 ±0.6	15.3 ±1.2	32
Klebsiella pneumonia **GN**	-	13.3 ±1.5	11.7 ±1.2	13.3 ±1.2	-	15.3 ±1.2	14.6 ±0.6	15.6 ±1.2	-	19.3 ±0.6	16.3 ±0.6	17.7 ±0.6	31
Candida albicans **FS**	11.7 ±1.5	-	12.0 ±1.0	10.6 ±0.6	15.7 ±0.6	12.3 ±0.6	16.3 ±0.6	13.3 ±0.6	18.0 ±1.0	15.3 ±0.6	18.7 ±0.6	14.6 ±0.6	35
Mucar recemosus **FS**	13.3 ±1.5	-	15.6 ±1.2	-	16.7 ±1.2	12.0 ±1.0	17.3 ±1.6	11.7 ±1.5	21.3 ±1.5	13.7 ±0.6	21.6 ±2.5	13.7 ±1.2	32
Rhizoctonia solani **FS**	13.7 ±0.6	-	13.0 ±1.0	-	15.3 ±0.6	-	15.3 ±1.2	13.3 ±1.5	17.3 ±1.6	-	17.7 ±1.2	15.7 ±0.6	34
Rhizopus stolonifer **FS**	13.3 ±1.2	-	13.7 ±0.6	12.3 ±0.6	15.6 ±1.2	-	16.7 ±1.2	14.6 ±0.6	17.3 ±1.6	13.3 ±0.6	21.3 ±0.6	15.6 ±1.2	30
Saccharomyces cerevisiae **FS**	14.3 ±0.6	15.3 ±1.5	13.7 ±1.2	13.7 ±0.6	15.6 ±1.2	14.6 ±0.6	16.7 ±1.2	15.7 ±0.6	17.7 ±0.6	21.3 ±0.6	21.0 ±1.0	19.3 ±0.6	29

Volume per well: 50µl; Borer size used: 6mm; **H**-Hexane, **C**-Chloroform, **M**-Methanol and **W**-Water.
GP = Gram positive; **GN** = Gram negative; **FS** = Fungal species and (-) indicates 'No inhibition'.
Diameter of zone of inhibition (mm) including disc diameter of 6mm: mean of three assays ± standard deviation.

CONCLUSION

Our study confirms that, the leaf and stem extracts of *H. curassavicum* possess antibacterial, antifungal activities. There is a possibility of developing this plant as a source of antibacterial and antifungal agent and further investigations are necessary to identify the bioactive principles. The variation of antimicrobial activity of present study might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

ACKNOWLEDGEMENTS

We thank Head of the Department of Pharmaceutical Sciences, Andhra University for their kind cooperation and valuable support to carry out this work. K. Prasanna Lakshmi is grateful to UGC SAP for the grant of Junior Research Fellowship.

REFERENCES

- Ferreira, M. J., Pinto, D. C., Cunha, Â., & Silva, H. (2022). Halophytes as medicinal plants against human infectious diseases. *Applied Sciences*, *12*(15), 7493.
- Arya, S. S., Devi, S., Ram, K., Kumar, S., Kumar, N., Mann, A., ... & Chand, G. (2019). Halophytes: The plants of therapeutic medicine. *Ecophysiology, abiotic stress responses and utilization of halophytes*, 271-287.
- Combs, C. A., & Anderson, H. (1949). Use of mangrove bark. *Australian Leather Trade Rev*, 43, 270-274.
- Patra, J. K., & Thatoi, H. N. (2011). Metabolic diversity and bioactivity screening of mangrove plants: a review. *Acta Physiologiae Plantarum*, *33*, 1051-1061.
- Falleh, H., Ksouri, R., Medini, F., Guyot, S., Abdelly, C., & Magne, C. (2011). Antioxidant Activity and Phenolic Composition of the Medicinal and Edible Halophyte *Mesembryanthemum edule* L. *Ind. Crop Prod*, 34, 1066-1071.
- Qadir, M. A., Shahzadi, S. K., Bashir, A., Munir, A., & Shahzad, S. (2017). Evaluation of Phenolic Compounds and Antioxidant and Antimicrobial Activities of Some Common Herbs. *Int J Anal Chem*, Id. 3475738, 1–6.
- Prasanna Laskhmi, K., & Narasimha Rao, G. M. (2013). Antimicrobial Activity of *Suaeda monoica* (Forsst ex Geml) against Human and Plant Pathogens. *Res J Pharm Bio Che Sci*, *4*(2), 680-685.
- Prasanna Lakshmi, K. (2015). Ecological and Antimicrobial Studies on Some Halophytic Species of Godavari Estuary. *Ph.D Thesis*, 1-192.
- Prasanna Laskhmi, K., & Narasimha Rao, G. M. (2023a). Antimicrobial Screening of the Solvent Extracts of Halophytic Plant *Suaeda maritima* (L.) Demort. Against Selected Pathogens, *Sch Acad J Biosci*, 11(9), 315-322.
- Prasanna Laskhmi, K., & Narasimha Rao, G. M. (2023b). Antimicrobial Activity of *Sesuvium portulacastrum* (L.) Against Selected Pathogens. *Haya: The Saudi J Life Sci*, *8*(9), 161-168.
- Adelaja, A. A., Ayoola, M. D., Otulana, J. O., Akinola, O. B., & Olayiwola, A. A. (2008). Evaluation of the Histo-gastroprotective and Antimicrobial Activities of *Heliotropium indicum* Linn. (Boraginaceae). *Malaysian J Med Sci*, *15*(3), 22-30.
- Reina, M., Gonzalez-Coloma A., Gutierrez, C., Cabrera, R., & Henriquez, J. (1997). Bioactive Saturated Pyrrololizidine Alkoloids from *Heliotropium floridum*. *Phytochem*, *46*(5), 845-853.
- Hussain, S., Jamil, M., Ullah1 F., Khan, A., Ullah, F., Arfan, M., Ahmad, S., & Khatoon, L. (2010). Antimicrobial and Antioxidant Activities of the Plant *Heliotropium strigosum*. *African J Biotechnol*, *9*(45), 7738-7743.
- Osungunna, M. O., & Adedeji, K. A. (2011). Phytochemical and Antimicrobial Screening of Methanol Extract of *Heliotropium indicum* Leaf. *J Microbiol & Antimicrobials*, *3*(8), 213-216.
- Ghori, M. K., Ghaffari, M. A., Hussain, S. N. Manzoor, M., Aziz, M., & Sarwer, W. (2016). Ethanopharmacological, Phytochemical and Pharmacognostic Potential of Genus *Heliotropium* L. / *Heliotropium* L. Cinsinin Etnofarmakolojik, Fitokimyasal ve Farmakognozik Potansiyeli. *Turkish J Pharm Sci*, 13(2): 259+.
- Narasimha Rao, G. M. (2012). Distribution pattern and present scenario of Mangroves and associated flora of Andhra Pradesh. Chapter 3 in; Biodiversity of Aquatic Resources. Published by Daya Publishing House, New Delhi. PP. 29-49.
- Umamaheswara Rao, M., & Narasimha Rao, G. M. (1988). Mangrove Populations of the Godavari Delta Complex. *Indian J Mar Sci*, 17, 326-329.
- Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., & Yolken, H. R. (1995). Manual of *Clinical Microbiol., 6th Edition, ASM Press, Washington DC*, 15-18.
- Olurinola, P. F. (1996). *A Laboratory Manual of Pharmaceutical Microbiology.* Idu Abuja, Nigeria, 69-105.