

In-vitro Assessment of Antibacterial and Electrochemical Properties of Methanolic Leaf Extracts of *Holigarna ferruginea* March. The Poisonous Plant Species in the Western Ghats

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Submission Date: 14-08-2022; Revision Date: 18-09-2022; Accepted Date: 11-10-2022.

ABSTRACT

Background: The *Holigarna ferruginea*. March. (Black varnish tree) is one of the plant species belonging to the family Anacardiaceae, it is endemic to the Western Ghats. The whole genus shows potent medicinal properties in human life. on that criterion, we took action **Aim:** To evaluate the antibacterial properties of methanolic leaf extract of *Holigarna ferruginea*. March. **Materials and Methods:** The chopped and shade-dried leaves were extracted using Soxhlet and methanol as solvent. Preliminary phytochemical screening revealed the presence of alkaloids, phenols, flavonoids cardiac glycosides, steroids, and tannins. The current research was carried out to investigate the antibacterial activity of *Holigarna ferruginea*. March. against Both Gram-positive and Gram-negative bacteria, pathogens whose many species are responsible for a wide range of diseases. And also studied scavenging properties by an electrochemical method (*Cyclic voltagramm*). **Results:** The methanolic leaf extract was tested for antibacterial activity using the well diffusion technique, in which the wells with varying concentrations of the extract were prepared and used. It was at a dosage of 5mg/ml, the methanolic extracts were more effective, with greater inhibition zones in *Salmonella enterica* (17.66±0.57 mm), *Staphylococcus aureus* (17.66±0.57 mm), *Escherichia coli* (17.66±0.57 mm/) and *Klebsiella pneumonia* (16.66±0.57), then the additional concentrations of 2.5, and 1.25mg/ml were reported comparatively less zone of inhibition in all pathogenic bacteria. From this stud the *Holigarna ferruginea*. March. had excellent efficacy against both Gram-positive and Gram-negative bacteria. The electrochemical method was evidenced and reported moderate scavenging properties and confirms the presence of free radicals in the extract. **Conclusion:** These findings suggest that *H. ferruginea* has antibacterial and antioxidant properties, making it a potentially useful antimicrobial phytomedicine. The reported activity backs up the plant's traditional use in the treatment of infectious diseases.

Keywords: *Holigarna ferruginea*, GC-MS, Antibacterial, Cyclic voltagramm.

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INTRODUCTION

Every woody or shrubby medicinal plant shows great medicinal and therapeutic properties, those plants are an integral part of human society, and traditional

holistic practices of medicine like Ayurveda, Siddha, Homeopathy, and Unani are formulated their formulations by herbal compounds.^[1] Plants are rich with illness-curing properties because of their bioactive metabolites, nearly about 80% of terrestrial flora is the source of navel secondary metabolites. Recently after the COVID-19 pandemic, In China average, 90% of the 214 patients recovered and improved their immunity through traditional herbal medicine treatment.^[2] The plant form Anacardiaceae member is Widely used as medicine for its antibacterial and antioxidant properties especially the whole genus *Holigarna* (allergic plant)

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DOI: 10.5530/ajbls.2022.11.90

causes contact dermatitis. However, because of their medicinal action, it is used to treat cancer, obesity, and skin diseases.^[3] *Holigarna* is one of the endemic tree genera in Western Ghats of India, it is a deciduous toxic plant containing acrid juice that belongs to the family Anacardiaceae, the genus *Holigarna* shows potentiality against the bacterial activity, Skin diseases, arthritis, hemorrhoids, and shows anti-cancerous, and antiseptic properties.^[4,5] likewise, *Holigarna caustic* has been reported and shows some properties to treat hemorrhoids, obesity, skin diseases, eye irritations, tumors, and cancer diseases, and it also acts as an anti-inflammatory.^[1] Based on the literature survey, the whole genus shows potential therapeutic properties from basic antimicrobial activity to anticancer activity, even though bioactive reports are in less number, for this reason, the present study aimed to investigate therapeutic uses as antibacterial, antioxidant the present study aimed to investigate therapeutic uses as an antibacterial, and antioxidant activity of methanolic leaf extract of *Holigarna ferruginea*.

MATERIALS AND METHODS

Sample Collection

The fresh leaves of *Holigarna ferruginea* from the region of Bisle Ghat (Karnataka), and the collected sample were transported to the laboratory in clean polythene bags, and the sample was examined and identified with the help of floras and monographs.

Preparation of Organic Solvent Extract

Samples were cleaned and shade dried for 72 hr, then plant materials were powdered using a mechanical grinder. The leaf powder was extracted using high polarity methanol solvent. Dried leaf powder of about 100 g was weighed, put in cheesecloth, and subjected to extract successively with 500 ml methanol in the Soxhlet extractor until the extract was clear. The extract was condensed and preserved in a refrigerator in air-tight vials for further use.

Preliminary Phytochemical Screening

The crude leaf methanolic extract of *Holigarna ferruginea* was tested for the presence of metabolites like Alkaloids, Glycosides, Phenols, Tannins, Cardiac glycosides, Terpenoids, Steroids, and Flavonoids. The results are expressed as '+' for the presence and '-' for the absence.^[6]

GC-MS Analysis

The methanolic extract was analyzed using GC-MS; first, the temperature was held at 30°C for approximately 2 min, and then it was raised to a maximum of 200°C.

The GCMS chromatogram of methanolic leaf extract of *Holigarna ferruginea* revealed a distinct peak with different retention times, a total of 70 different compounds are reported. The distinct peaks of known phytochemicals are comparatively identified by the National Institute of Standards and Technology (NIST05) database library.^[7]

Antibacterial Screening

Test Organism and Culture Media

In vitro antimicrobial activity of methanol extract of *Holigarna ferruginea* was tested against laboratory control strains of the Microbial Type Culture Collection (MTCC) taken from the IMTECH Chandigarh. Antibacterial activity was evaluated against one gram-positive and three gram-negative bacteria. The gram-positive bacteria used were: *Staphylococcus aureus* MTCC (3160), the gram-negative bacteria utilized in the assay were: *Escherichia coli* MTCC (42), *Klebsiella pneumonia* MTCC (7407), *Salmonella enterica* MTCC (3231) by the Muller-Hinton agar well diffusion method (MHA, Hi-media, India). On the surface of MHA plates, 100 µl of CFU/ml diluted inoculums of various bacterial strains were swabbed and given enough time to solidify. A cork borer was used to prepare the well, which was then filled with 45 mL of plant extract in three different concentrations (5, 2.5, and 1.25 mg/mL), and Ampicillin was used as positive control and allowed for the incubation of bacterial growth at 37°C and zone of inhibition was measured (mm) All triplicates' The statistics were reported as the mean of all the experiments taken.

Electrochemical method for Antioxidant activity by CV

The Antioxidant activity of *Holigarna ferruginea* was carried out by using a Cyclic voltameter the electrochemical method here the electrochemical activity of the selected methanolic extract was determined by Electrochemical workstation CHI 60°C, the measurements were carried out using a conventional three-electrode system namely the saturated calomel as a reference electrode, platinum wire as a counter electrode and glossy corban paste electrode as a working electrode. The 0.5 g of the extract was dissolved in 100 ml of methanol for analysis, and 0.2M phosphate buffer with pH 7.0 was used as the electrolyte. The cyclic voltammetric behavior of quiring simple was recorded at a different scan rate of electric potential from 50 – 300 mV/s.^[10]

RESULTS

Alkaloids, tannins, flavonoids, cardiac glycosides, steroids, and phenols were found in the preliminary

phytochemical examination of the methanolic leaf extract of *Holigarna ferruginea*. The findings of this investigation were presented in (Table 1). The findings

are shown as a plus sign (“+”) for the presence and a negative sign (“-”) for the absence.

GC-MS analysis of Methanol leaf extract of *Holigarna ferruginea*

A phytochemical profile with 70 different compounds was evidenced after the Gas chromatography-mass spectrometry (GC-MS) results of Methanol leaf fractions of *Holigarna ferruginea* (Figure 1). Among 70 bioactive phytochemical compounds, about 11 compounds are listed in (Table 2). Based on the maximum range of Retention time and Peak area.

The antibacterial activity of *Holigarna ferruginea* exhibited a great zone of inhibition against the tested pathogenic bacterial strains shown in (Figure 2), and statistical data are shown in (Table 3). About 5mg/mL of methanolic extract of the leaf shows a maximum zone of inhibition against *Staphylococcus aureus*, *Salmonella enterica*, and *Escherichia coli* which is 17.66 ± 0.57 mm, and the minimum zone of inhibition shown in *Klebsiella pneumoniae* which is 16.66 ± 0.5 mm. likewise, the standard drug (Ampicillin) reported a 32.66 ± 1.15 mm zone of inhibition in *Salmonella enterica*, 31.33 ± 0.57 mm in *Staphylococcus aureus*, 27.33 ± 1.52 mm in *Escherichia*

Table 1: Preliminary Phytochemical analysis of methanolic leaf extract of *Holigarna ferruginea*.

Phytochemical tests	Result	Phytochemical tests	Result
Alkaloids	+	Phenols	+
Flavonoids	+	Steroids	+
Tannins	+	Terpenoids	-
Glycosides	-	Cardiac glycosides	+

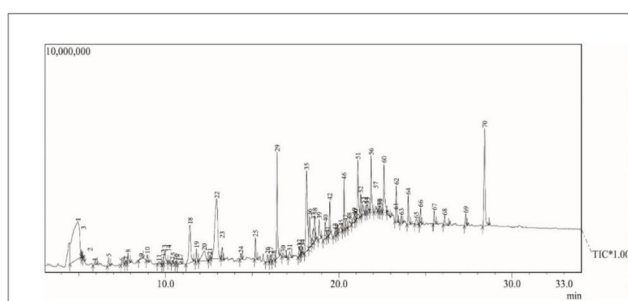


Figure 1: GC-MS Profile of methanol leaf extract of *Holigarna ferruginea*.

Table 2: Major bioactive profile of *Holigarna ferruginea*.

Sl. No.	RT	Area	Peak area %	Compound	Molecular formula	Molecular Weight	Application	Reference
1	12.952	31935238	10.59	(1R, 3R, 4R,5R,)- (-) Quinic acid	C ₇ H ₁₂ O ₆	192	Antibacterial, antiviral, antioxidant	[8]
2	16.438	18870182	6.26	n- Hexadecenoic acid	C ₁₇ H ₃₄ O ₂	256	Antioxidant, nematicide, pesticide	[9]
3	18.139	22753571	7.54	9-Octadecenoic acid, 1,2,3-propanetriyl ester	C ₅₇ H ₁₀₄ O ₆	884	Anti-spasmodic and immune modulators	[10]
4	2 1.101	10103959	3.535	Hexatriacontane	C ₃₆ H ₇₄	506	Antioxidant	[11]
5	28.408	20335100	6.74	Gamma. -Sitosterol	C ₂₈ H ₅₂ O ₂	432.7	Anticancer	[12]
6	18.315	6559446	2.18	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5	Antioxidant	[13]
7	12.234	6565794	2.18	Xylitol	C ₅ H ₁₂ O ₅	152	Anti-cancer, anti-inflammatory	[14]
8	15.192	3144299	1.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	Antimicrobial and Anti-inflammatory	[15]
9	18.613	4026210	1.34	Docosane	C ₂₂ H ₄₆	310.6	Antibacterial	[16]
10	18.867	3704389	1.23	9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	Anti-inflammatory, Antibacterial, Antiarthritic	[11]
11	20.128	2078126	2.69	Dotriacontane	C ₃₂ H ₆₆	450.9	Antimicrobial, antioxidant, antispasmodic	[17]

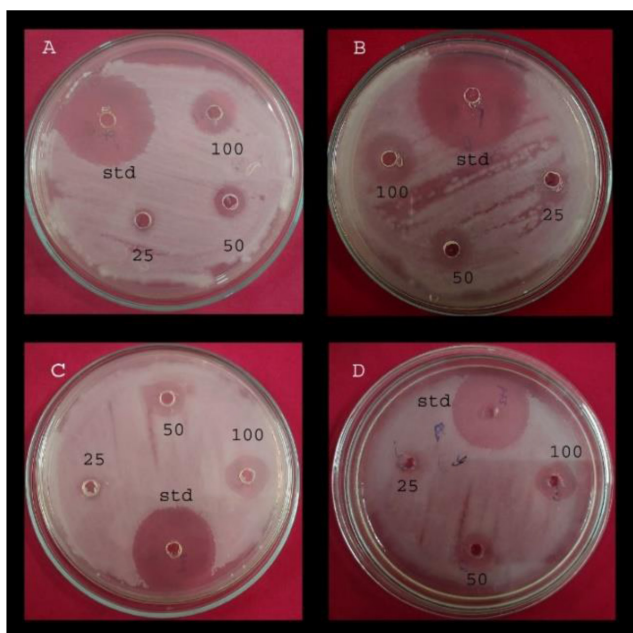


Figure 2: The antibacterial activity of methanolic leaf extract of *Holigarna ferruginea* against A- *Klebsiella pneumonia* MTCC (7407), B- *Staphylococcus aureus* MTCC (3160), C- *Escherichia coli* MTCC (42), D- *Salmonella enterica* MTCC (3231) * std-Standard (Ampicillin) (100% - 5, 50% - 2.50, 25% - 1.25).

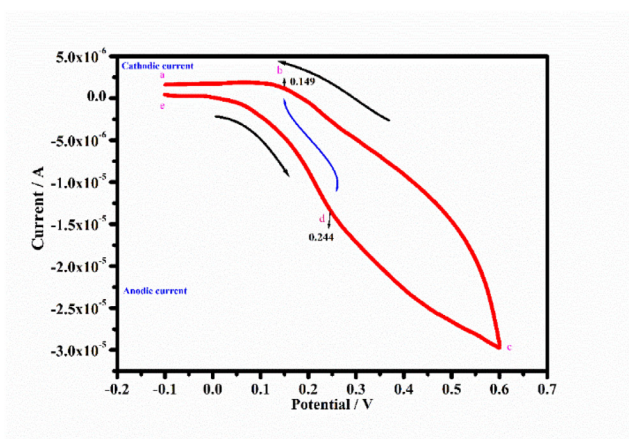


Figure 3: Cyclic voltammogram of methanolic leaf extract of *Holigarna ferruginea*. March.

coli, and about 26.66 ± 1.52 mm in *Klebsiella pneumoniae* at 1 mg/mL concentration.

The present work revealed that in methanolic extract of *Holigarna ferruginea* under the influence of anodic and cathodic current the reduction process occurs from (a) the initial potential to (c) due to the potential scanning the reduction curve was formed (b) at 0.149 V (cathodic current i_{pc}), due to positive potential scanning in anodic current the oxidation curve was formed (d) at 0.244 v (anodic current i_{pa}) shown in (Figure 3).

DISCUSSION

In the present study, the investigation of bioactivity properties of a methanolic leaf extract from *Holigarna ferruginea* revealed the presence of various metabolites, including alkaloids, Phenols, flavonoids, tannins, steroids, and cardiac glycosides. These bioactive metabolites are responsible for therapeutic actions.^[7] The GC-MS analysis is one of the best and most used techniques for the separation of phytoconstituents and reveals the presence of 70 different phytochemical compounds, based on the high retention peak area about 11 compounds are listed. The n- Hexadecenoic acid, Docosane, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, plays a vital role and shows antibacterial, antioxidant, and anti-inflammatory properties.^[15,16] The Gamma- γ -Sitosterol, 9,12-Octadecadienoic, Xylitol, and were found to be cytotoxic against cancer cell lines (MCF7).^[11,12,14] In general, Gram-positive bacteria appear to be more sensitive to the inhibitory effects of plant extracts than Gram-negative bacteria. Gram-positive bacteria are susceptible due to their single-layered cell wall structure, whereas the Gram-negative cell wall is multi-layered and quite complex.^[18] In our study, the *Holigarna ferruginea* reported good activity in both Gram-positive as well as Gram-negative bacteria like *Salmonella enterica* (17.66 ± 0.57 mm), *Staphylococcus aureus* (17.66 ± 0.57 mm), *Escherichia coli* (17.66 ± 0.57 mm) and *Klebsiella pneumonia*

Table 3: The antibacterial activity of methanolic leaf extract of *Holigarna ferruginea* against pathogenic microorganisms.

Sl. No.	Concentration in mg/mL	Zone of inhibition (mm) (Mean of three replicates \pm standard deviation)			
		<i>Salmonella enterica</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
1	Standard	32.66 \pm 1.15	31.33 \pm 0.57	27.33 \pm 1.52	26.66 \pm 1.52
2	5 (100%)	17.66 \pm 0.57	17.66 \pm 0.57	17.66 \pm 0.57	16.66 \pm 0.57
3	2.50 (50%)	12.66 \pm 0.57	13.66 \pm 0.57	15.33 \pm 0.57	14.33 \pm 0.57
4	1.25 (25%)	9 \pm 1	9.66 \pm 0.57	8.33 \pm 0.57	7 \pm 0

*Concentration (5, 2.50, 1.25 mg/mL), *Standard (Ampicillin) 1mg/ml.

(16.66±0.57), here the *Klebsiella pneumonia* shows less sensitivity towards crude as well as standard antibacterial drug ampicillin. The degree of antibacterial activity of flavonoids is substantially determined by the freedom of the attached phenolic groups. Likewise, the free phenolic groups exhibit stronger antibacterial action than their substituted counterparts.^[19] However, there is little research on the biological potential of *H. ferruginea* from India. In comparison to our findings, Pradeep and Saj demonstrated the antibacterial activity of *H. arnotiana* from Kerala. According to the previous report, the antibacterial effect of *H. arnotiana* may possibly be due to the presence of several chemical components like alkaloids, steroids, tannins, phenolic compounds, flavonoids, steroids, resins, fatty acids, and gums.^[5] The cyclic voltameter has been predominantly used for the investigation of the reduction, Oxidation, and antioxidant properties of biological samples.^[20] In our study, the cyclic voltammety graph shows the presence of both oxidative and reductive active components in the solution mixture, at 0.149 V (cathodic current i_{pc}) and 0.244 v (anodic current i_{pa}) and it confirms the plant extract is consistent with the Scavenger analysis and the electrochemical approach. Due to the electric scanning in the anodic and cathodic fields, the oxidation and reduction peaks developed at various scan rates. The scavenging activity was observed by the positive potential scanning in an anodic current. The shifting of the oxidation curve or anodic peak potential towards positive values indicates the presence of scavenging oxygen free radicals in the biological samples or extract.^[21]

CONCLUSION

These findings indicate that *H. ferruginea* has antibacterial and antioxidant characteristics, which represents a standardizing antimicrobial phytomedicine. The reported activity supports the plant's traditional usage in the management of infectious illnesses. The examined extract and some of the analysed components may help in the creation of novel antibacterial medications. This hypothesis will, however, be supported by the toxicological and pharmacological analysis of the targeted samples.

ACKNOWLEDGEMENT

The authors are very thankful to Kuvempu University, Shivamogga providing R&D lab facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

H. ferruginea: *Holigarna ferruginea*; **mg:** Milligram; **mm:** millimetre; **g:** gram; **GC-MS:** Gas chromatography-mass spectrometry; **+**: presence: **-:** absence; **hr:** hour; **mL:** milliliter; **°C:** degree Celsius; **NIST:** National Institute of Standards and Technology; **MTCC:** Microbial Type Culture Collection; **µl:** micro liter; **m:** minute; **pH:** potential of Hydrogen; **RT:** Retention Time; **%:** Percentage; **Sl No:** serial number; **std:** standard; **V:** Volt. **R&D:** Research and Development.

Authors' Contributions

K Manjunath has done the research under the supervision of Prof. Y. L. Krishnamurthy

SUMMARY

Holigarna ferruginea is native to the Western Ghats. The entire genus has therapeutic characteristics thus, we evaluated the antibacterial activities of *Holigarna ferruginea* leaf. Alkaloids, phenols, flavonoids, cardiac glycosides, steroids, and tannins were found in the phytochemical screening. The current study investigated the antibacterial activity of *Holigarna ferruginea*. against Gram-positive and Gram-negative bacteria. Electrochemically studying scavenging characteristics (Cyclic voltagramm). The antibacterial activity of the methanolic leaf extract was evaluated using the well diffusion method and reported a good zone of inhibition in all pathogenic bacteria. Both Gram-positive and Gram-negative bacteria were susceptible to *Holigarna ferruginea* extract. The electrochemical approach showed moderate free radical scavenging and confirmed the extract's existence.

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Cite this article: Manjunath KM, Krishnamurthy YL. *In-vitro* Assessment of Antibacterial and Electrochemical Properties of Methanolic Leaf Extracts of *Holigarna ferruginea* March. The Poisonous Plant Species in the Western Ghats. *Asian J Biol Life Sci.* 2022;11(3):684-9.