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IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF THE ISOLATION COMPOUNDS RUTIN, FERULIC ACID, OLEANOLIC ACID AND STIGMASTEROL OF ALL PART OF HELICTERES ISORA

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ABSTACT

The Present study investigates the isolation and characterization of rutin, ferulic acid, oleanolic acid and stigmaterol of all part of Helicteres Isora and aim to prove rutin, ferulic acid, oleanolic acid and stigmaterol as a antioxidant and antibacterial agents that can be develop a new chemotherapy as antibiotics. *Helicteres Isora* is belongs to family *sterculiacae* is distributed widely in forests throughout India and commonly known as East Indian screw tree is a medicinally important sub- deciduous shrub or asmall tree. It has been used in traditional medicine for treatment of various diseases and disorders like skin problems dermatitis eczema acne gastropasm. The in vitro antioxidant and antibacterial activities of the isolated compounds rutin, ferulic acid, oleanolic acid, and stigmasterol were systematically evaluated. In the DPPH radical scavenging assay, all compounds exhibited concentration-dependent antioxidant activity (20–100 µg/ml), with oleanolic acid (IC_{50} = 12.543 µg/ml) showing activity closest to the standard ascorbic acid (IC_{50} = 11.005 µg/ml), followed by ferulic acid (19.305 µg/ml), rutin (25.697 µg/ml), and stigmasterol (30.227 µg/ml). The reducing power assay further confirmed dose-dependent antioxidant potential, with increasing absorbance at 700 nm indicating enhanced reducing capability, comparable to the standard ascorbic acid. In the antibacterial study using the agar well diffusion method, all isolated compounds demonstrated significant activity against both Escherichia coli and Staphylococcus aureus. Oleanolic acid exhibited the highest activity against E. coli (29±1.732 mm), while rutin showed the strongest inhibition against S. aureus (32.333±0.577 mm). Overall, the findings suggest that the isolated phytoconstituents possess notable antioxidant and antibacterial properties, supporting their potential therapeutic applications.

KEYWORD : Helicteres Isora, Antioxidant and Antibacterial activities, DPPH redical scavenging activity of ascorbic acid, rutin, ferulic acid, oleanolic acid and stigmaterol.

1. INRTODUCTION

Helicteres isora is belongs to family *Sterculiacae*, commonly known as East Indian screw tree, is a medicinally important, sub-deciduous shrub or small tree of reaching a height of 5- 15feet. Almost all parts of the plant are

used in traditional medicinal system for curing various diseases.[1-2] It has been used in traditional medicine for treatment of various diseases and disorders like skin problems dermatitis eczema acne gastropasm.It is used as an antispasmodic, antipyretic,anti-diarreoheal anti-dysentric,for tapeworms and as a tonic after childbirth.the root juice is claimed to be useful in treating cough,asthma diabetes, emphyenma,intestinal infection, snake bites and a cure for scabies when applied topically [3-4]. The root and stem barks are considered to be expectorant, demulcent, astringent and anti-galactagogue and are useful in colic, scabies, empyema, gastropathy, diabetes, diarrhoea and dysentery. The fruits were also found to possess significant antispasmodic activity In the present investigation.

Antimicrobial assay of both isolated compounds of Ferulic acid F(H), Oleanolic acid F(D), Stigmasterol F(F) and Rutin F(E) (2mg/ml) was performed by agar well diffusion method in Nutrient agar media (NAM) plates. For the preparation of NAM Media, 28 g of Nutrient Media was dissolved in 1 liter of distilled water, and the pH was verified before autoclaving at 121°C and 15 lbs pressure for 15 minutes. Subsequently, the NAM media was poured into plates and allowed to solidify in a laminar air flow. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. Next, 100µl of the inoculum was transferred to a fresh sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, and four wells of 6 mm was bored into the inoculated media. Each well was then filled with varying samples (F(H), F(D), F(F) and F(E)). After 30 minutes of diffusion at room temperature, the plates were incubated at 27°C for 24 hours. The antibacterial activity was determined by measuring the diameter (mm) of the clear zone of growth inhibition**.** It was found that purified rutin ferulic acid oleanolic acid and stigmaterol has antioxidant and antibacterial activity.

2. MATERIAL AND METHODS

2.1 In vitro anti oxidant activity

2.1.1 DPPH assay

Free radical scavenging activity of the rutin, ferulic acid, oleanolic acid and stigmasterol, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of **Ali et al (2013)**. Different volume of extracts/standard (20 – 100µg/ml) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated by using the equation:

% Scavenging activity=[(A0-A1)/A0]×100

Where A0 is the absorbance of the control and A1 is the absorbance of the extract. Lower the absorbance, the higher is the free radical scavenging activity. The curves were prepared and the IC50 value was calculated using linear regression analysis.

2.1.2 Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100 µg/ml.

Preparation of extracts

Stock solutions of extract were prepared by dissolving 10 mg of dried extracts in 10 ml of desired solvent (water or organic solvent) to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and extract (20 to 100µg/ml) in

1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) tri-chloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Shimadzu-1700). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard **(Choi et al., 2002).**

2.2 In vitro Antimicrobial activity

2.2.1 Bacterial strains and culture conditions

The bacteria used in this study were *E. coli* MTCC 42, *S. aureus* MTCC 10787.

2.2.1 Antimicrobial activity by well diffusion assay

Antimicrobial assay of both isolated compounds of Ferulic acid F(H), Oleanolic acid F(D), Stigmasterol F(F) and Rutin F(E) (2mg/ml) was performed by agar well diffusion method in Nutrient agar media (NAM) plates. For the preparation of NAM Media, 28 g of Nutrient Media was dissolved in 1 liter of distilled water, and the pH was verified before autoclaving at 121°C and 15 lbs pressure for 15 minutes. Subsequently, the NAM media was poured into plates and allowed to solidify in a laminar air flow. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. Next, 100µl of the inoculum was transferred to a fresh sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, and four wells of 6 mm was bored into the inoculated media. Each well was then filled with varying samples (F(H), F(D), F(F) and F(E)). After 30 minutes of diffusion at room temperature, the plates were incubated at 27°C for 24 hours. The antibacterial activity was determined by measuring the diameter (mm) of the clear zone of growth inhibition**.**

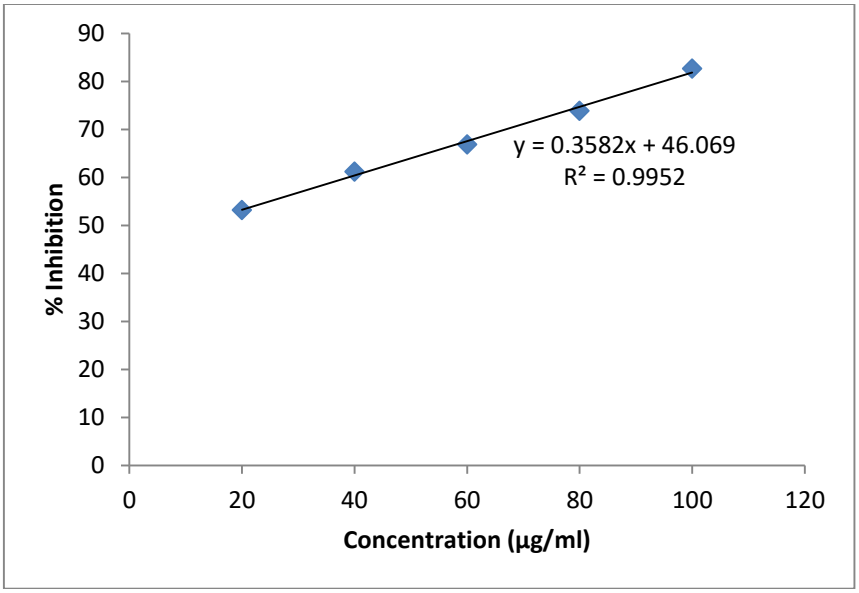
3. RESULTS

3.1 In vitro anti oxidant activity

3.1.1 DPPH Assay

Table 1 DPPH radical scavenging activity of Ascorbic acid

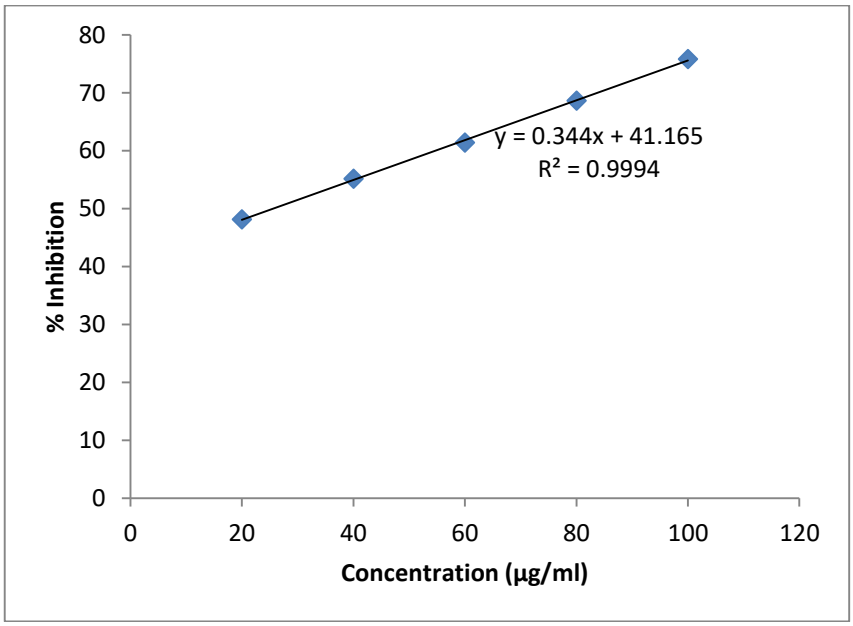
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.462	53.19149
40	0.383	61.19554
60	0.327	66.8693
80	0.258	73.86018
100	0.171	82.67477
Control	0.987	
IC50	11.005	



Graph 1 Graph represent Concentration of Ascorbic acid Vs Absorbance

Table 2 DPPH radical scavenging activity of Rutin

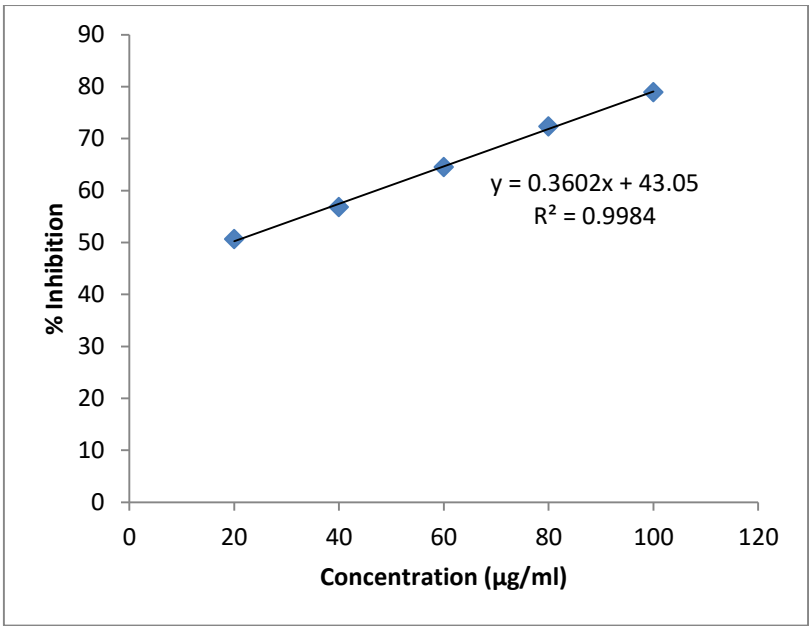
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.512	48.12563
40	0.443	55.11651
60	0.381	61.39818
80	0.31	68.59169
100	0.239	75.78521
Control	0.987	
IC50	25.697	



Graph 2 Graph represent Concentration of Rutin Vs Absorbance

Table 3 DPPH radical scavenging activity of Ferulic acid

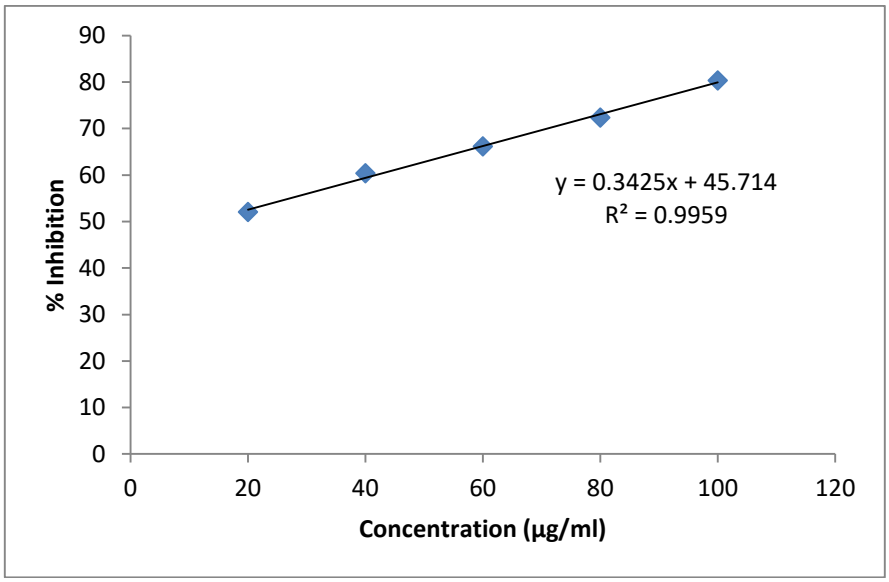
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.487	50.65856
40	0.426	56.83891
60	0.35	64.53901
80	0.273	72.34043
100	0.208	78.92604
Control	0.987	
IC50	19.305	



Graph 3 Graph represent Concentration of ferulic acid Vs Absorbance

Table 4 DPPH radical scavenging activity of Oleanolic acid

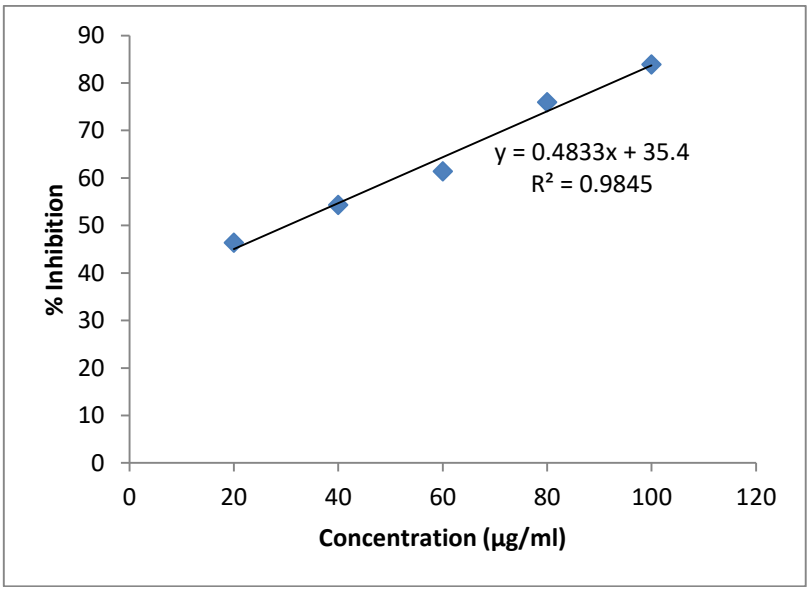
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.473	52.077
40	0.391	60.38501
60	0.334	66.16008
80	0.273	72.34043
100	0.194	80.34448
Control	0.987	
IC50	12.543	



Graph 4 Graph represent Concentration of Oleanolic acid Vs Absorbance

Table 5 DPPH radical scavenging activity of Stigmasterol

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.529	46.40324
40	0.451	54.30598
60	0.381	61.39818
80	0.237	75.98784
100	0.159	83.89058
Control	0.987	
IC50	30.227	

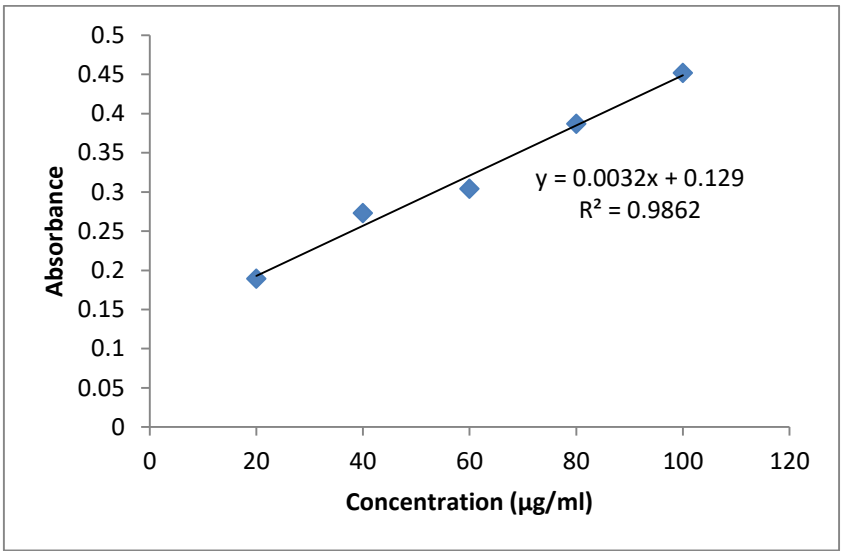


Graph 5 Graph represent Concentration of Stigmasterol Vs Absorbance

3.1.2 Reducing Power Assay

Table 6 Reducing power assay of Ascorbic acid

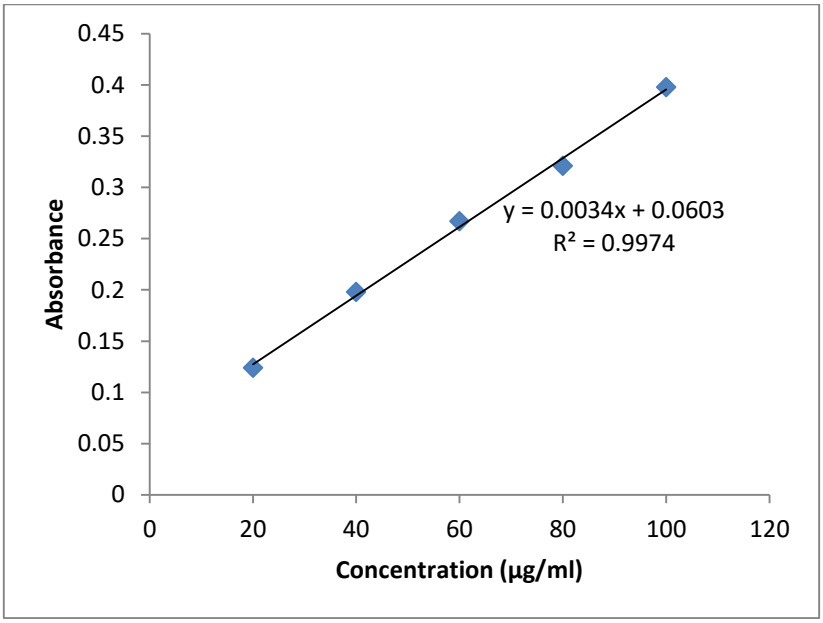
Concentration (µg/ml)	Absorbance
20	0.189
40	0.273
60	0.304
80	0.387
100	0.452



Graph 6 Graph represent Concentration of Ascorbic acid Vs Absorbance

Table 7 Reducing power assay of Stigmasterol

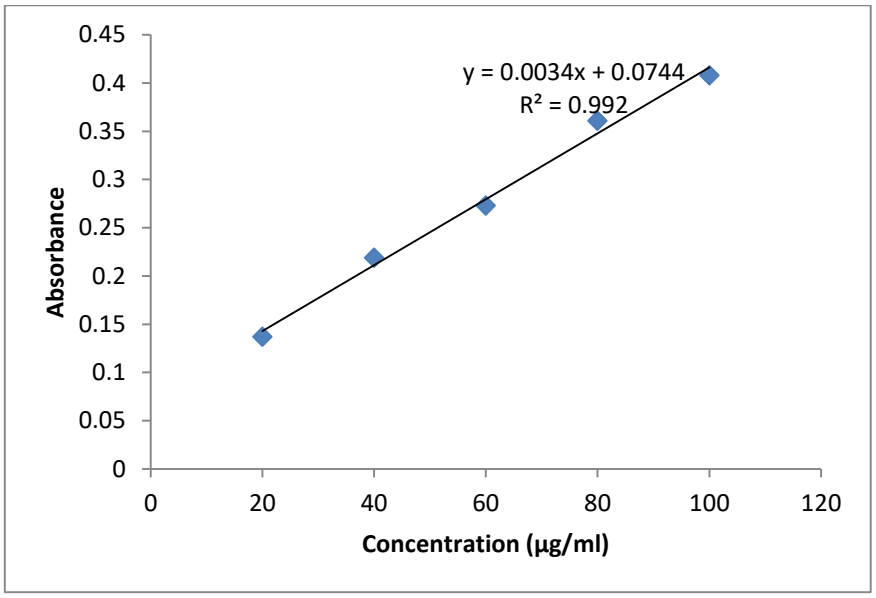
Concentration (µg/ml)	Absorbance
20	0.124
40	0.198
60	0.267
80	0.321
100	0.398



Graph 7 Graph represent Concentration of Stigmasterol Vs Absorbance

Table 8 Reducing power assay of Rutin

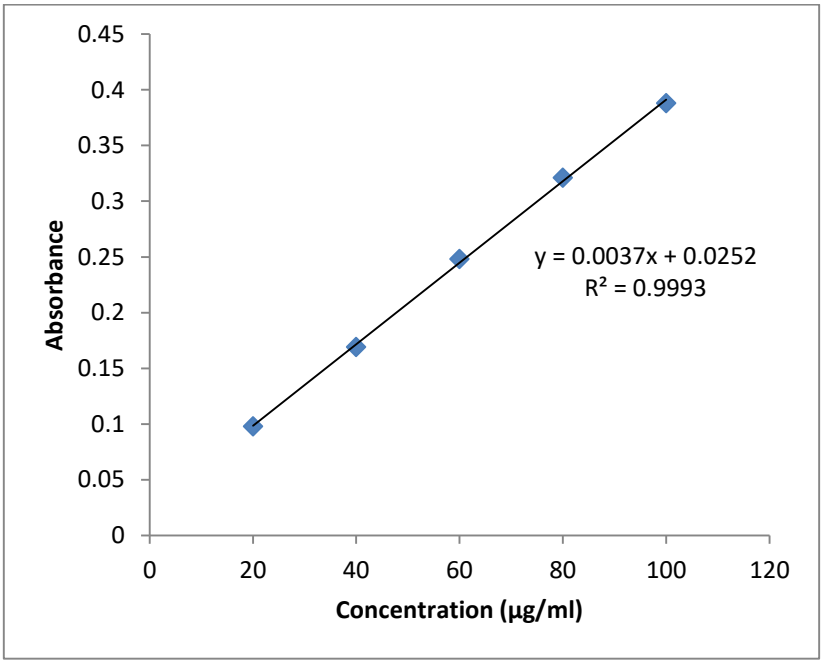
Concentration (µg/ml)	Absorbance
20	0.137
40	0.219
60	0.273
80	0.361
100	0.408



Graph 8 Graph represent Concentration of Rutin Vs Absorbance

Table 9 Reducing power assay of Ferulic acid

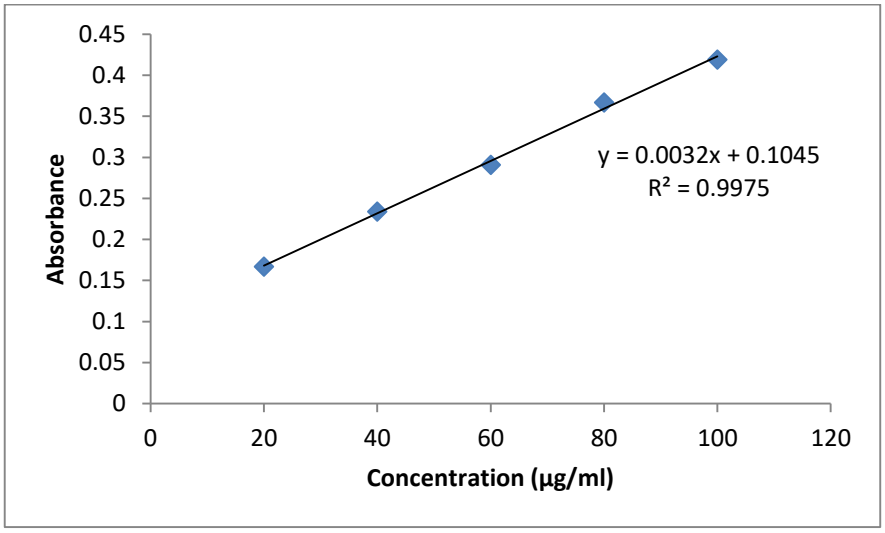
Ferulic acid	
Concentration (µg/ml)	**Absorbance**
20	0.098
40	0.169
60	0.248
80	0.321
100	0.388



Graph 9 Graph represent Concentration of Ferulic acid Vs Absorbance

Table 10 Reducing power assay of Oleanolic acid

Olenolic acid	
Concentration (µg/ml)	**Absorbance**
20	0.167
40	0.234
60	0.291
80	0.367
100	0.419



Graph 10 Graph represent Concentration of Oleanolic acid Vs Absorbance

3.2 In-vitro anti bacterial activity

The *in-vitro* antibacterial potential of the isolated compounds, namely Ferulic acid, Oleanolic acid, Stigmasterol and Rutin was evaluated against a panel of Gram-positive and Gram-negative bacterial strains using the agar diffusion method. The selected microorganisms included *Escherichia coli*, and *Staphylococcus aureus* representing clinically and biologically significant pathogens.

Table 11 Antibacterial activity of isolated compounds against *Escherichia coli*

Sample	Zone of inhibition in mm			
	Plate 1	Plate 2	Plate 3	Mean±SD
Ferulic acid F(H)	26	27	28	27±1
Oleanolic acid F(D)	30	30	27	29±1.732
Stigmasterol F(F)	29	29	25	27.666±2.309
Rutin F(E)	28	28	27	27.666±0.577

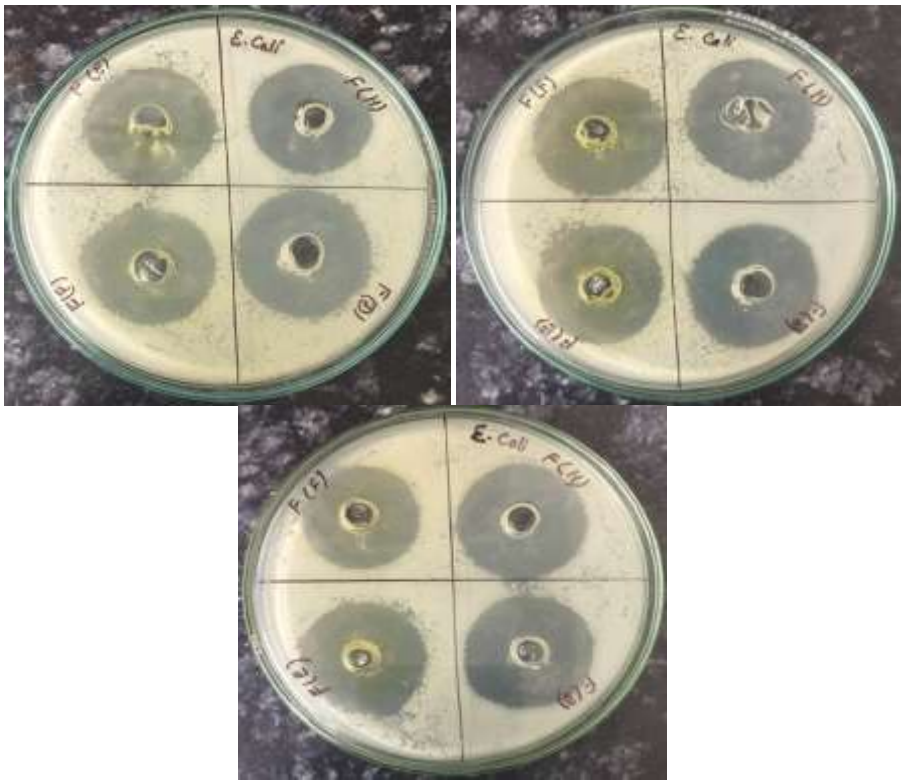


Figure 1 Antibacterial activity of isolated compounds against *Escherichia coli*

Oleanolic acid demonstrated moderate antibacterial activity against *Escherichia coli*, showing a mean inhibition zone of 29±1.732mm, whereas Stigmasterol and Rutin showed activity about 27.666±2.309mm and 27.666±0.577mm. Ferulic acid has the lowest 27±1mm activity among all the compounds.

Table 12 Antibacterial activity of isolated compounds against *Staphylococcus aureus*

Sample	Zone of inhibition in mm			
	Plate 1	Plate 2	Plate 3	Mean±SD
Ferulic acid F(H)	29	30	33	30.666±2.081
Oleanolic acid F(D)	31	31	31	31±0
Stigmasterol F(F)	30	30	30	30±0
Rutin F(E)	32	33	32	32.333±0.577

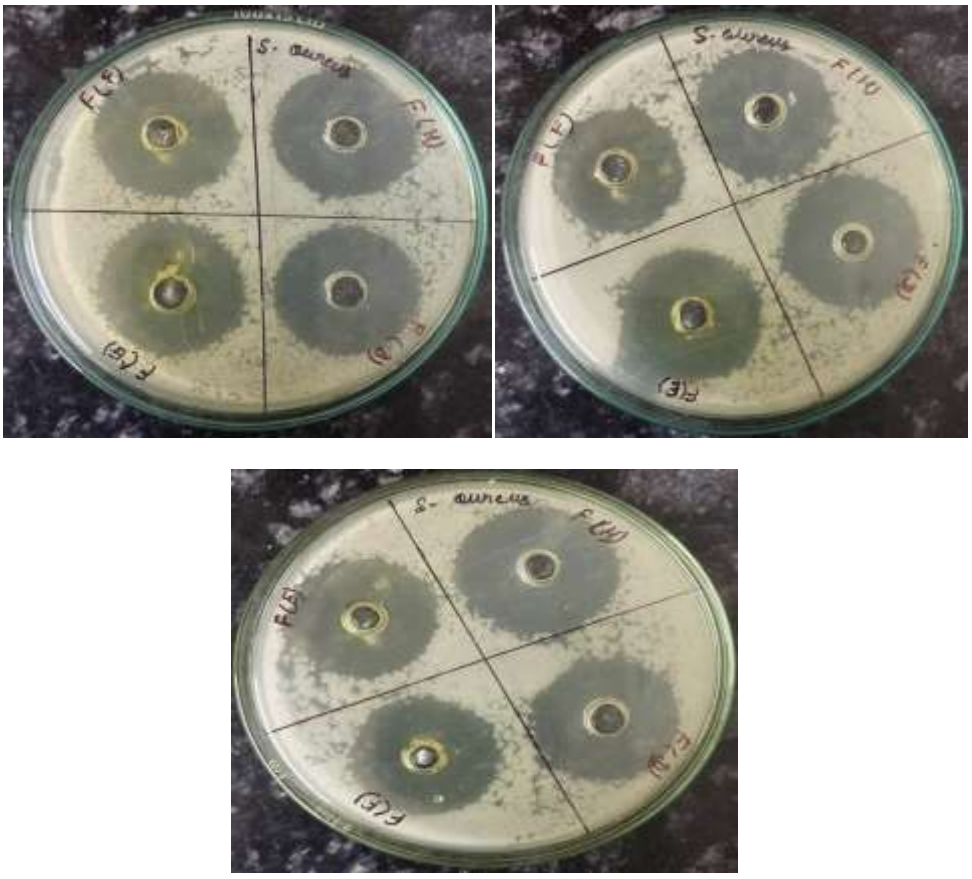


Figure 2 Antibacterial activity of isolated compounds against *Staphylococcus aureus*

Rutin demonstrated moderate antibacterial activity against *Staphylococcus aureus*, showing a mean inhibition zone of 32.333±0.577mm, whereas Oleanolic acid, Ferulic acid and Stigmasterol showed activity about 31±0mm, 30.666±2.081mm and 30±0mm.

4 .CONCLUSION

The studies reveals that Rutin shows remarkable antimicrobial activities against important human pathogens which is identified The *in-vitro* antibacterial potential of the isolated compounds, namely Ferulic acid, Oleanolic acid, Stigmasterol and Rutin was evaluated against a panel of Gram-positive and Gram-negative bacterial strains using the agar diffusion method. The selected microorganisms included *Escherichia coli*, and *Staphylococcus aureus* representing clinically and biologically significant pathogens. Oleanolic acid demonstrated moderate antibacterial activity against *Escherichia coli*, showing a mean inhibition zone of 29±1.732mm, whereas Stigmasterol and Rutin showed activity about 27.666±2.309mm and 27.666±0.577mm. Ferulic acid has the lowest 27±1mm activity among all the compounds. Rutin demonstrated moderate antibacterial activity against *Staphylococcus aureus*, showing a mean inhibition zone of 32.333±0.577mm, whereas Oleanolic acid, Ferulic acid and Stigmasterol showed activity about 31±0mm, 30.666±2.081mm and 30±0mm. However, still more scientific evaluation and clinical trials are required to establish its therapeutic efficacy.

5. REFERENCES

1. Kirtikar KR, Basu BD. An ICS. 1. *Indian medicinal plants*. 2nd ed. Allahabad, India: Lalit Mohan Basu; 1981.
2. Kumae D, Singh RK, Farooq S, *In vitro* Antimicrobial Activity of Oleanolic acid Identified in Chloroform Extract of Fruits of *Helicteres isora L*. World Journal of Pharmaceutical Research, 2016; 6(1): 1102-1112.
3. Venkatesh S, Laxmi KS, Reddy BM, Ramesh M. 3. Antinociceptive activity of *Helicteres isora*. *Fitoterapia*, 2007; 78: 146-8.

4. Singh K, JK: Ethnobotany of *Helicteres isora Linn*. In Kheri district, Uttar Pradesh. J. Economic Taxonomic Botany1985; 7(2): 487-492.
5. Kirtikar KR Indian Medicinal Plants, Dehradun, India: International Book distributors 1995;(1): 371-372.
6. Ali, M. S., Amin, M. R., Kamal, C. M. I., & Hossain, M. A., In vitro antioxidant, cytothrombolytic activities and phytochemical evaluation of methanol extract of the A. philippense L. leaves. Asian Pacific Journal of Tropical Biomedicine 2013. 3(6): 464-469.
7. Choi CW,Kim SC, Hwang SS, Choi BK, Ahn HJ. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants flavonoids by assay-guided comparison. *Plant Sci*. 2002;163:1161-8.
8. Manandhar, S., Luitel, S., & Dahal, R. k. (2019). *In vitro* Antimicrobial Activity of some Medicine plants against human pathogenic bacteria. *Journal of tropical medicine*, 2019.
9. Loganayaki N, Antioxidant activity and free radical scavenging capacity of phenolic extract from *Helicteres isora L*. And Ceiba pentandra L. J Food Sci technol 2013;DO1.10.1007/S 1397-011-0389-x.
10. Sochor J, Fully automated spectrophotometric protocols for determination of antioxidant activity: Advantages and Disadvantages. Molecules. 2010; 15: 8618-8640.
11. Sultana B, Effect of extraction solvent/technique on the Antioxidant activity of selected medicinal plant extracts Molecules. 2009; 14: 2167-2180.
12. K. Horiuchi, S. Shiota, T. Hatano, T. Yoshida, T. Kuroda and T. Tsuchiya,"Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compoundson vancomycin-resistant enterococci (VRE)," *Biological & Pharmaceutical Bulletin*, 2007; 30(6): 1147–1149.
13. S. Fontanay, M. Grare, J. Mayer, C. Finance, and R. E. Duval, "Ursolic, oleanolic and betulinic acid: antibacterial spectra and selectivity indexes," *Journal of Ethnophaemacology*, 2008; 120(2): 272-276.