International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 30-06-2021, Accepted: 16-07-2021, Published: 31-07-2021
Volume 6, Issue 4, 2021, Page No. 501-505

Antibacterial and antioxidant activity of *Hydnocarpus alpinus* Wight (Achariacea)

J Mariyaraj
Research Project Coordinator, Xavier Research Foundation (XRF), St. Xavier'S College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India

Abstract
The present investigation deals with the antimicrobial and antioxidant study of methanolic and acetone leaf extract of *Hydnocarpus alpinus*. It plays a vital role in the traditional medicine system for ever. The oil from its seeds has been widely tested and used in day today life but the leaf does not get much aware of. This study mainly focused on leaf extract and its medicinal value. Eight pathogenic bacteria's were taken for test. Among them six pathogenic bacteria showed good inhibition which means the plant extract inhibit the pathogenic bacteria' s growth and the IC_{50} value of methanolic and acetone leaf extract showed that it had effective antioxidant activity, so it could provide a significant bioresource of antioxidants in pharmaceutical industry.

Keywords: *Hydnocarpus alpinus*, antioxidant assay, antimicrobial activity, endemic species

Introduction
Hydnocarpus Gaertn is an Indo-Malasian genus belonging to the family Achariaceae According the APG 4 System of classification. Five species of *Hydnocarpus viz*., *H. alpina*, *H. kurzii*, *H. macrocarpa*, *H. pentandra and H. pendulus* are reported from India. Out of the five species, *H. macrocarpa*, *H. alpine and H. pendulus* are endemic to South India. *H. pentandra* is the most widely distributed species The 'Chaulmoogra' oil, which is used for the treatment of leprosy, is extracted from the seeds of *H. Kurzii* and other species of *Hydnocarpus viz, H. alpina* and *H. pentandra* (David *et. al.*, 2014) [4].
It is threatened by habitat loss. Traditional healers have been used *Hydnocarpus* species for centuries to treat various diseases. Different parts of *H. alpinus* have been used for the treatment of various human ailments such as itches, cuts, swellings, eczema. Hence the current study was designed to evaluate the antimicrobial activity test against eight pathogenic bacteria and antioxidant activity of leaf extracts of *H. alpinus* by using radical scavenging, antioxidant assay and Nitric oxide activities and determination of total ascorbic content.
However, the perusal of literature has revealed that no studies have so far been undertaken to explore the antioxidant and antimicrobial activity in the leaves of *H. alpinus*. Hence the present study aims to analyze the antioxidant and antimicrobial activity in the methanolic and acetone extract of the leaves of *H. alpinus*

Materials and Methods
Collection and Authentication
The plant was collected from Ooty nearby Halakarai, Tamilnadu India, during April 2018. The plant was identified by Dr. S. John Britto, Director and Head, The Rapinat Herbarium and Center for Molecular Systematic St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen (No: 68237) was deposited at the Rapinat Herbarium.

Fig 1: *H. alpinus* twig with matured fruit and the collected locality

Extraction Procedure
Collected leaf of plant were dried at room temperature, powdered, and was then stored in air tight container till use. It was weighed in a selected quantity and was subjected to soxhlet apparatus using solvents such as Acetone, Aqueous, Ethanol and Methanol respectively. The solvent was then evaporated to get dry Powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of antioxidant and antibacterial studies.

Antioxidant Activity
Total antioxidant capacity assay
The total antioxidant capacity assay was determined as described (Prieto, 1999) [13]. Different concentrations of the ethanolic and methanolic leaf extracts of *H. alpinus* (10-50µg/ml) were taken and added 1.0 ml of the reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid. The antioxidant activity is calculatedas follows:

Antioxidant activity (%) = (A0 – A1 / A0) X 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.

DPPH Radical Scavenging activity
Radical scavenging activity was measured by using DPPH scavenging method of (Blois, 1958) [1]. A solution of DPPH in methanol (24µg/ml) was prepared and 2ml of this solution was added to ethanol and methanol bark extract at different concentrations (10- 50µg/ml). Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Ascorbic acid was used as standard. The percentage of inhibition was calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.

Nitric oxide scavenging assay
Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan, 2003) [6]. The Ethanolic and Methanolic bark extracts of *S. febrifuga* were added to different test-tubes in varying concentrations (10-50 µg/ml). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make up volume to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% Sulphanilamide, 0.1% Naphthylethylenediamine dichloride and 3% Phosphoric acid) was added to each test tube. The absorbance was measured immediately at 546 nm. The percentage of scavenging activity was measured with reference to ascorbic acid and the inhibition was calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where; A0 is the absorbance of control and A1 is the absorbance of test

Antimicrobial activity
Test Micro-organisms
Eight bacterial species collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, Punjab, India were used in this study. The microbial strains used are *Escherichia coli* (MTCC # 119), *Klebsiella pneumonia* (MTCC # 3040), *Serratia marcescens* (MTCC # 2645), *Staphylococcus aureus* (MTCC # 3163), *Bacillus cereus* (ATCC # 4342), and *Streptococcus faecalis* (ATCC # 7066). All the test bacterial strains were maintained on nutrient agar media at 4 °C.

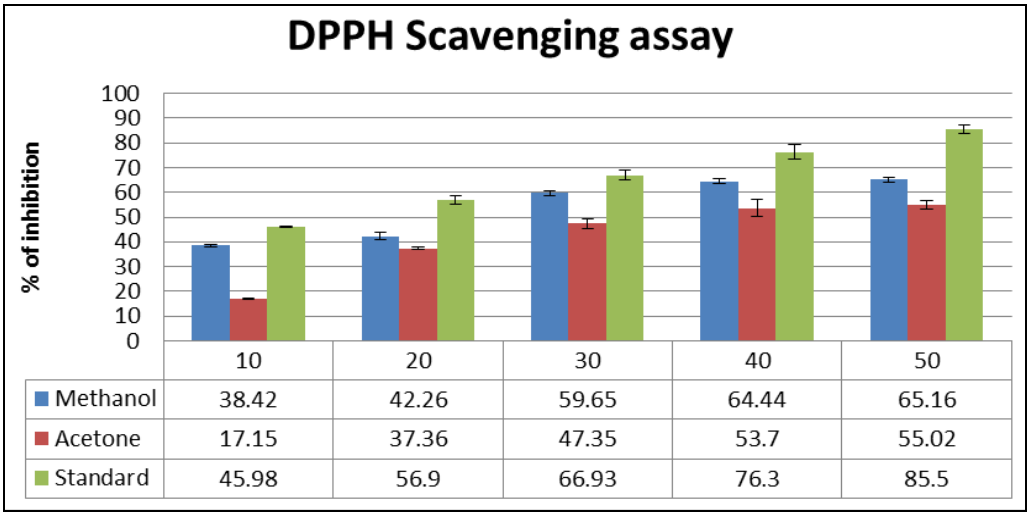
Preparation of Disc
6 mm discs were prepared and sterilized in autoclave and were soaked in different extracts like Acetone, Distilled water, Ethanol and Methanol. The standard drug streptomycin was used as control.

Determination of Antibacterial Activity
Antibacterial activities of the *H. alpinus* extract were determined by disc diffusion method. Nutrient agar was prepared for the study. Each plate of Nutrient agar was swabbed with each bacterial strain by using sterile cotton swab. The soaked dried discs were placed on the surface of each inoculated plate. The plates were allowed for diffusion for half an hour and then transferred to incubator at 370c for 24 hours. Standard disc of Streptomycin was also placed as positive control. The antibacterial activity of *H. alpinus* whole plant extract was determined by measuring the diameter of zone of inhibition in mm.

Result
In the present investigation of *H. alpinus* had been selected on the basis of their traditional practices for various diseases. Methanolic and acetone leaf extract of the selected plant was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay and nitric oxide scavenging assay.

Antioxidant assays
The antioxidant activity of plant materials were assayed by employing the following methods:

DPPH radical scavenging activity
In the present study, the antioxidant capacity of *H. alpinus* leaf extract of methanol and acetone were evaluated using DPPH radical scavenging method by comparing with the standard ascorbic acid activity as a known antioxidant. In this experiment, the concentrations range from 10-50µg/ml and highest percentage of inhibition was in methanol extract 65.16% at 50 µg/ml. (Fig. 2)

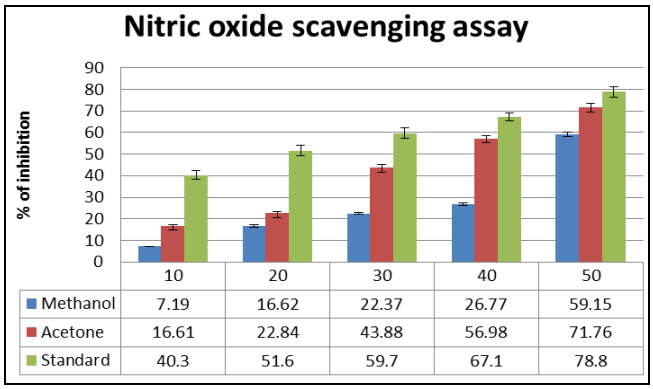


	10	20	30	40	50
Methanol	38.42	42.26	59.65	64.44	65.16
Acetone	17.15	37.36	47.35	53.7	55.02
Standard	45.98	56.9	66.93	76.3	85.5

Fig 2: DPPH Scavenging assay of leaf extract of methanol and acetone *H. alpinus* compared to that of Ascorbic acid. Each value is expressed as mean ± standard deviation (n=3).

Nitric oxide

Nitric Oxide (NO) scavenging assay is based on the scavenging ability of *H. alpinus* as well as ascorbic acid as standard. Maximum inhibition of NO was observed in the methanol and acetone extracts highest concentration (50µg/ml) for the samples. At this maximum concentration, inhibition was found to be 71.76% (acetone) for ascorbic acid, which serves as the standard. (Fig. 3)

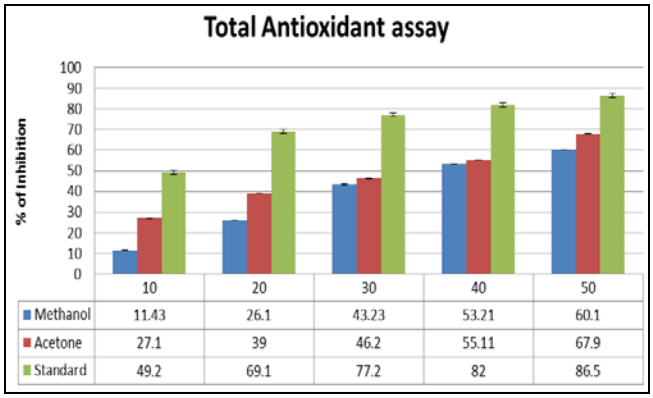


	10	20	30	40	50
Methanol	7.19	16.62	22.37	26.77	59.15
Acetone	16.61	22.84	43.88	56.98	71.76
Standard	40.3	51.6	59.7	67.1	78.8

Fig 3: Nitric oxide scavenging of leaf extract of *H. alpinus* compared to that of Ascorbic acid. Each value is expressed as mean ± standard deviation (n=3).

Total antioxidant activity

The total antioxidant assay of *H. alpinus* was determined by phosphormolybdenum using standard Ascorbic acid. In phosphormolybdenum assay, the concentrations range from 10- 50µg/ml. it showed higher percentage of activity of acetone as 67.09% at 50µg/mL. (Fig. 4)



	10	20	30	40	50
Methanol	11.43	26.1	43.23	53.21	60.1
Acetone	27.1	39	46.2	55.11	67.9
Standard	49.2	69.1	77.2	82	86.5

Fig 4: Total antioxidant assay of leaf extract of *H. alpinus* compared to that of Ascorbic acid. Each value is expressed as mean ± standard deviation (n=3).

Antibacterial assays of *H. alpinus* in the leaf (Table 1)

The results obtained from antibacterial screening showed the highest antibacterial activity on acetone extract followed by methanol, ethanol and aqueous extracts, the following Table 1, Chart 1, and Plate 1. Which explains the extract values and its significance.

Table 1

S.No	Test microorganism	Inhibition Zones(mm)				
		Acetone	Methanol	Ethanol	Aqueous	Standard
1.	*Bacillus cereus*	0	0	0	0	7±1
2.	*Escherichia coli*	0	0	0	12±1	22.6±2.08
3.	*E. faecalis*	12.33±0.57	13.66±2.06	13.76±3.78	0	19.33±2.08
4.	*K. oxytoca*	0	0	8.66±1.15	7.67±0.57	14±1
5.	*K. pneumoniae*	8.33±0.57	9±1	12±1	9.33±0.57	18±1
6.	*S. aureus*	0	16.6±1.54	9.66±1.52	0	22±0.57
7.	*S. lantus*	7.33±0.57	10.66±0.57	17±1	17.32±1.52	23.66±2.08
8.	*S. marcescens*	8±0.57	10±1	14±1	8±1	16.66±1.52

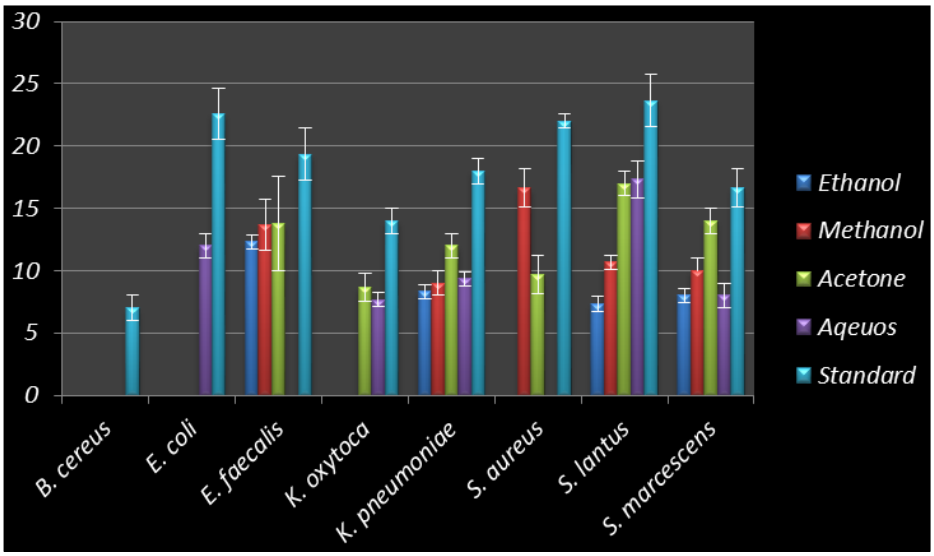


Chart 1: Antibacterial assays of *H. alpinus* in the leaf

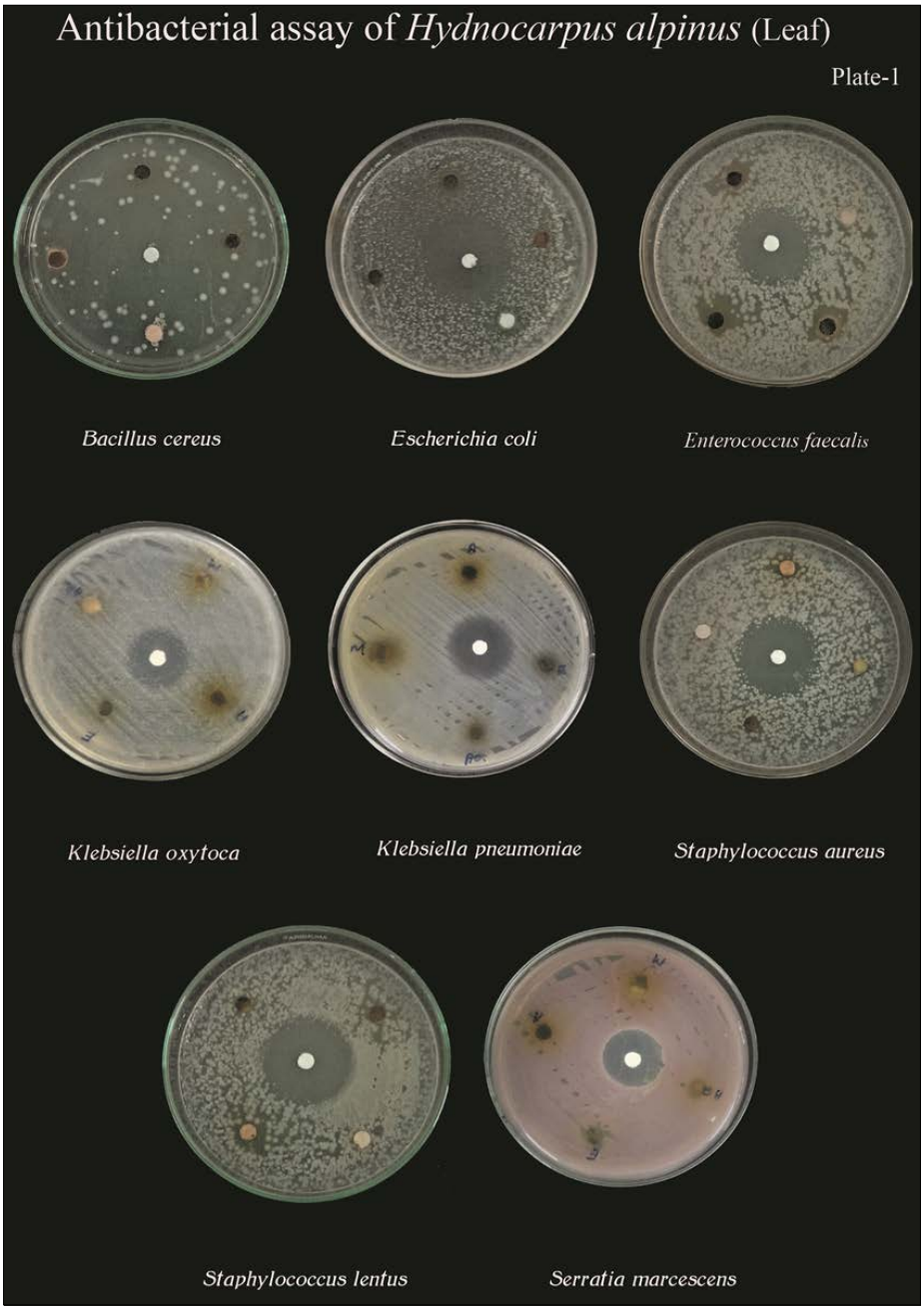


Plate 1: Antibacterial assays of *H. alpinus* in the leaf

Conclusion

The present study reveals that the Antibacterial activity on the leaf extract of *H. alpinus* exhibited high inhibition against the Pathogenic bacteria likely *E. faecalis, K. oxytoca, K. pneumoniae and S. marcescens*. The sample was subjected to specific tests of the antioxidant assay using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay and nitric oxide scavenging assay. The results have distinctly proved the antioxidant potential of *H. alpinus* and promising for the treatment of human society. The diversity of Pharmacological activity in the *H. alpinus* makes this plant a potential source for further pharmacological investigation.

Acknowledgment

The Author is thankful to Dr. V Anand Gideon research guide, Associate Professor and Head of The Department of Botany, Bishop Heber College (Autonomous) Tiruchirappali. He is also indebted to Director and Head, The Rapinat Herbarium and Center for Molecular Systematic, St. Joseph's College (*Autonomous*) for providing necessary facilities

References

1. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature,1958:181:199-1200.
2. Cinda Cicily Varghese, Bhat AR, Sandhya S, Kavitha MP, Krishnakumar K. *Hydnocarpus Wightiana*: A Phytopharmacological Review International Journal Of Natural Products Research,2015:5(2):17-19.
3. CAMP Workshops on Medicinal Plants, India. *Hydnocarpus macrocarpa subsp. macrocarpa*. The IUCN Red List of Threatened Species, 1998.
4. David T, George KV. HPTLC analysis of the leaf extract of *Hydnocarpus pendulus* Manilal, Sabu & Sivarajan. Int. J. Pharm. Life Sci,2014:5(4):3452-3462.
5. Dhanasekaran M, Karuppusamy M, Annadurai M, Rajasekaran KM. Evaluation of the Phytochemical Constituent of Indian Medicinal Plant *Hydnocarpus alpina* Wight. Indian J. Pharm. Biol. Res,2013:1(3):23-28.
6. Govindarajan R, Rastogi S, Vijayakumar Metal. Studies on antioxidant activities of *Desmodium gangeticum*. Bio Pharm Bull,2003:26:1424.
7. Joshi Arun B, Harijan Kiran C. Physicochemical and Phytochemical Investigation of the Roots of *Hydnocarpus pentandrus* (Buch.-Ham.) Oken International Journal of Pharmaceutical Sciences Review and Research Rev. Res Article No. 44,2014:25(1):260-265.
8. Jacobsen PL, Levy L. Mechanism by which hydnocarpic acid inhibits mycobacterial multiplication. Antimicrobial agents and Chemotherapy,1973:3:373.
9. Karunamoorthi K, Jegajeevanra SarifulIsam Md. Study of Pharmacological Activities of Methanolic Extract of *HydnocarpusKurzii* Bark Submitted Department of Pharmacy East West University 9th February, 2016.
10. Krishnan SM, Dhanalakshmi P, Sudhalakshmi YG, Gopalakrishnan S, Manimaran A, Sindhu S. Evaluation of phytochemical constituents and antioxidant activity of Indian medicinal plant *Hydnocarpus pentandra*. International Journal of Pharmacy and Pharmaceutical Sciences,2013:5(2):453-458.
11. Manilal KS, T Sabu, VV Sivarajan. A New Species *Hydnocarpus Gaertn.* (Flacourtiaceae) from India. Trop. Plant Sci.Res,1983:1(4):355.
12. Prashith Kekuda TR, Vinayaka KS. Inhibitory activity of *Harpulliaarborea* (Blanco) Radlk. And *Hydnocarpuspentandra* (Buch.-Ham.) Oken against seed-borne fungi Journal of Medicinal Plants Studies,2017:5(4):114-117.
13. Prieto P, Pineda M, Aguilar M. Spectrophometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to determination of vitamin E. Anai Biochem,1999:269:337-341.
14. Ramana P, Dhathri NR. Biochemical Characterization Of *Hydnocarpus pentandra* For Seed Oil Quality, Oil Cake And Its Biodiesel Production Potential College Of Agriculture, University Of Agricultural Sciences, Dharwad.
15. Shirona TK, Sruthy KB, Rajendran N. Antibacterial and antioxidant properties of two medicinal plants *Hydnocarpus pentandra* and *Eupatorium triplinerve* from Kerala, India.
16. Sivaraman G, Paulraj GM, Gandhi RM, Reegan DA, Ignacimuthu S. Larvicidal potential of *Hydnocarpus pentandra* (Buch.- Ham.) Oken seed extracts against *Aedes aegypti* Linn. and Culex quinquefasciatus Say (Diptera: Culicidae). International Journal of Pure and Applied Zoology,2014:2(2):109-112.