IJPSR (2019), Volume 10, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 July 2018; received in revised form, 18 October 2018; accepted, 20 October 2018; published 01 April 2019

EVALUATION OF ANTIMICROBIAL, ANTHELMINTIC AND ANTIOXIDANT ACTIVITIES OF *HOPEA PONGA* (DENNST.) BARK

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Keywords:

Antimicrobial, Anthelmintic, Antioxidant, *Hope ponga*, DPPH, ABTS, *Pheretima posthuma*

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ABSTRACT: The present work involves the extraction of phytochemicals from the bark of the medicinal plant *Hopea ponga*, with various solvents and evaluation of their antimicrobial, anthelmintic and antioxidant activities by following standard methods. The antioxidant capacity of the plant extracts was measured by their ability to scavenge free radicals such as (a) DPPH (2,2-diphenyl-1-picrylhydrazyl), (b) ABTS (2,2'-azinobis-(3-ethylbenzenothiaziline-6-sulfonate), (c) FRAP (Ferric Reducing Antioxidant Power) (d) Hydrogen peroxide assay. The methanol extract exhibited, high antiradical activity against DPPH, ABTS with an IC_{50} value of 19.48 µg/ml, 25.22 µg/ml, respectively, higher content of phenols (195.11 ± 2.14 mg expressed as mg of Catechol equivalents/100 mg dried extract and highest anthelmintic activity among all tested extracts. The ethyl acetate extract exhibited high antiradical activity against hydrogen peroxide with an IC_{50} value of 38.00 µg/ml and exhibited significant activity against *Streptococcus aureus* with a maximum inhibition zone of 35.93 ± 0.12 mm for 1000 mg/ml. The present study suggests that the methanol extract may contain active compounds, which have effective antimicrobial, anthelmintic and antioxidant activity.

INTRODUCTION: Plants have become the basis of traditional medicine system throughout the world for thousands of years and continue to provide mankind with new remedies. Also, plant-based drugs remain an important source of therapeutic agents because of their availability, relatively cheaper cost and non-toxic nature, when compared to modern medicine [1]. The use of anti-bacterial compounds to treat infection leads to the evolution of microbes resistant to existing drugs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(4).1838-47

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(4).1838-47

The emergence of resistance to the major classes of antibacterial agents is recognized as a serious health concern. The search for antibacterial agents with a new mode of actions will always remain as an important and challenging task. Plants based antimicrobial have enormous potential; they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with antimicrobials [2].

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause an enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia [3].

The gastrointestinal helminths become resistant to currently available anthelmintic drugs; therefore there is a foremost problem in the treatment of helminth diseases [4]. Hence, there is an increasing demand towards natural anthelmintics. Many medicinal plants contain active chemical constituents with high antioxidant property which plays an essential role in the prevention of various degenerative diseases [5]. Antioxidants are vital substances, which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. Reactive oxygen species are major sources of primary catalysts that create oxidative stress which results in numerous diseases and disorders. Thus, there is a need for more effective, less toxic and cost-effective antioxidant. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants [6].

The genus *Hopea ponga* (Dennst.), which belongs to the family Dipterocarpaceae, comprises 90 species, most of which are distributed in Southeast Asia till now only a few species have been investigated. This family of plants is known to produce a variety of Resveratrol oligomers [7, 8, 9, 10, 11, 12, 13]. These structures are very interesting and showed a wide range of biological activity, such as antibacterial, anticancer, antihepatotoxic, and anti-HIV. Resveratrol, a stilbene based phytochemical, is substantially helpful for human health owing to its significant anticancer, antioxidative, anti-HIV, antimicrobial, and long-microcirculation improving actions. Its oligomers have also received intense medical attention for their promising anticancer, antioxidant and antifungal potentials. *Hopea ponga*, endemic to Western Ghats of India, has not been chemically investigated to date. In the continuous effort to search for structurally new and biologically active metabolites from plants, we have carried out antimicrobial, anthelmintic and antioxidant activities of petroleum ether, ethyl acetate and methanol extracts of *Hopea ponga* stem bark.

MATERIALS AND METHOD:

Collection and Identification of Plant Material: The bark part of the *Hopea ponga* plant was collected from Nerale, Muthinakoppa region, N.R Pura, Chickmagalur, Karnataka, India, during April 2013. The plant was authenticated by taxonomist Dr. M. S. Pushpalatha, Rtd. Professor, Department of Botany, Sahyadri Science College, Shivamogga and a voucher specimen (SSC159) was deposited in the Department of Studies and Research in Chemistry, Sahyadri Science College (Autonomous), Shivamogga- 577203, Karnataka, India, for further reference.

Processing and Extraction: The bark of the plant was shade dried, coarsely powdered (2.5 kg) and was successively extracted with petroleum ether, ethyl acetate and methanol using a Soxhlet apparatus by hot extraction method. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was preserved in an airtight bottle. The crude extracts thus obtained were stored at 4 ºC for further investigation of potential antimicrobial, anthelmintic and antioxidant properties.

General Chemicals and Instruments: All chemicals and solvents used in the study were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2-Deoxy-D-ribose, Butylated Hydroxy Toluene (BHT), Folin-Ciocalteu's reagent was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), EDTA, ascorbic acid, ferric chloride was obtained from Merck (Darmstadt). Ascorbic acid, phosphoric acid, aluminum chloride, methanol are procured from Sd fine Chem. Ltd., India. All other chemicals and reagent used were of analytical grade.

Preliminary Phytochemical Screening: Standard phytochemical screening tests were performed to identify the different phytochemical constituents present in petroleum ether, ethyl acetate and methanol extracts of the plant [14, 15].

Antimicrobial Activity:

Organisms and Culture Media: The bacterial strains used to assess the antimicrobial property of the crude extracts of *Hopea ponga* bark were *Streptococcus aureus, Basilis subtilis, Bacillus cereus, Enterobactere aerogenes, Proteus vulgaris,* and *Klebsiella pneumonia*. Stock cultures were maintained at 4 °C on the slant of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth for bacteria

that were incubated for 24 h at 37 °C. The assay was performed by the Agar well diffusion method.

Screening for Antibacterial Activity:

Agar Well Diffusion Method: Agar well radial diffusion technique was used for the assessment of the antibacterial activity of the test samples. The sterilized nutrient agar medium was poured into sterilized Petri dishes. Nutrient broth containing 100 µl about 24 h old cultures of respective bacterial strains were spread separately on the agar medium. Wells were made using a stainless steel sterilized cork borer under aseptic conditions. 25, 50, 100 µg/ml of petroleum ether, ethyl acetate and methanol crude extracts were loaded into corresponding wells. The antibiotic Tetracycline was used as standard (1 µg/ml of sterile water). The plates were incubated for 24 h at 37 °C, and the diameter of the zone of bacterial growth inhibition was measured, and the readings were recorded in millimeter [16]. The tests were carried out in triplicates, and the results were recorded as mean ± SEM (Standard Error Mean). The antibacterial activities were evaluated by the determination of minimum inhibitory concentration (MIC) by micro broth dilution assay [17].

Evaluation of Anthelmintic Activity: The standard Albendazole (25 mg/ml) and the test solutions of *Hopea ponga* (Dennst.), (50, 100, 200 mg/ml) were evaluated for anthelmintic activity with Indian adult earthworm *Pheretima posthuma*. Observations were made for the time taken for paralysis and death of individual worms was recorded up to four hours of the test period. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for the death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water of 50 °C [18]. The tests were carried out in triplicates, and the results were recorded as mean ± SEM (Standard Error Mean).

***In-vitro* Antioxidant Assay:** The antioxidant activity of plant extracts was determined by the In vitro methods, (a) DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, (b) ABTS (2,2'-azinobis-(3-ethylbenzenothiaziline-6-sulfonate) assay, (c) FRAP (Ferric Reducing Antioxidant Power) assay, (d) Hydrogen peroxide assay.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity: DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al*. [19] The different concentrations of each of the extracts were prepared in methanol and were added to 3 ml of 0.1 mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in the dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition } (\%) = [(Ac\text{-}At) / Ac] \times 100$$

Ac: Absorbance of the control
At: Absorbance of the extracts/standard.

2, 2'- azino- bis (3- ethylbenzothiazoline- 6- sulphonic acid) (ABTS) Assay: ABTS radical scavenging activity of the extract was determined according to Re *et al*. [20] The $ABTS^{\cdot+}$ cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The plant extracts at various concentrations with 1ml of ABTS solution was homogenized, and its absorbance was recorded at 734 nm. Ethanol blanks were run with each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of the standard group was obtained by mixing 950 µl of $ABTS^{\cdot+}$ solution and 50 µl of BHT. As for the antiradical activity, ABTS scavenging ability was expressed as IC_{50} (µg/ml). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging activity } (\%) = [(Ac\text{-}At) /Ac] \times 100$$

Ac: Absorbance of the control
At: Absorbance of the extracts/standard.

Ferric Reducing Antioxidant Power (FRAP) Assay: The ability to reduce ferric ions was measured using the method described by Benzie and Strain [21]. The FRAP reagent was generated by

mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) TPTZ solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Plant extracts at different concentrations (20, 40, 60, 80 and 100 µg/ml) were then added to 3 ml of FRAP reagent, and the reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as mM $FeSO_4$ equivalents per gram of sample.

Reducing power (%) = [(Ac-At) / Ac] × 100

Ac: Absorbance of the control
At: Absorbance of the extracts/standard.

Hydrogen Peroxide Scavenging Activity: The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch *et al*. [22] A solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The plant extracts at different concentrations in 3.4 ml phosphate buffer were added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm.

Hydrogen peroxide scavenging activity (%) = [(Ac-At) /Ac] × 100

Ac: Absorbance of the control
At: Absorbance of the extracts/standard.

Estimation of Total Phenolic Content: The total phenolic content of the *Hopea ponga* (Dennst.), bark extract was determined by using Folin-Ciocalteu's reagent following a slightly modified method of Ainsworth [23]. Catechol was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the plant extract (100 µg/ml) was mixed with 2 ml of the Folin-Ciocalteu's reagent (diluted 1:10 with de-ionized water) and was neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using double beam UV-VIS spectrophotometer. A calibration curve was constructed using Catechol solution as standard, and total phenolic content of the extract was expressed in terms of milligrams of Catechol per gram of dry weight of the extract.

Statistical Analysis: Results are expressed as the standard error means of three independent experiments. Student's t-test was used for statistical analysis; P values > 0.05 were considered to be significant.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: Preliminary phytochemical analysis of various solvent extracts such petroleum ether, ethyl acetate and methanol extract of *Hopea ponga* (Dennst.), indicated the presence of certain secondary metabolites. Petroleum ether extract showed the presence of phytoconstituents alkaloids, flavonoids, tannins / phenolics, steroids/ triterpenoids, and saponins. While ethyl acetate and methanolic extracts showed the presence of phytoconstituents such as flavonoids, tannins / phenolics, steroids / triterpenoids, and saponins. The results of the phytochemical analysis are as shown in **Table 1.**

TABLE 1: IT SHOWS PHYTOCHEMICAL SCREENING OF VARIOUS SOLVENT EXTRACTS OF *HOPEA PONGA* (DENNST.), BARK

Phytoconstituents	Extracts		
	Pet ether	Ethyl acetate	Methanol
Alkaloids	+ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Tannins/Phenolics	+ve	-ve	+ve
Steroids/Triterpenoids	+ve	+ve	+ve
Saponins	+ve	+ve	-ve

Antibacterial Activity: The antibacterial activity was determined by measuring the diameter of the zone of inhibition recorded. The different extracts of the plant *Hopea ponga* (Dennst.), bark were found to have maximum antibacterial activity. The results of the antibacterial activity of different extracts against some bacterial strains are depicted in **Table 2A, 2B** and **2C** for petroleum ether, ethyl acetate and methanol respectively.

All three extracts of *Hopea ponga* (Dennst.), bark *viz*., petroleum ether, ethyl acetate, and methanol showed promising antibacterial activities against almost all bacterial strains.

In the present work, petroleum ether extract exhibited significant activity against *Klebsiella pneumonia* with an inhibition zone of 19.17 ± 0.12 mm. Ethyl acetate extract exhibited significant activity against *Streptococcus aureus* with an inhibition zone of 35.17 ± 0.13; methanol extract showed substantial activity against *Bacillus cereus* with an inhibition zone of 28.00 ± 0.09 mm for 1000 mg/ml of extract respectively.

The MIC values of the plant extract against the tested bacterial isolates ranged from 100 to 500 µg/ml **Table 3**. The methanol extract at the least concentration of 100 µg/ml inhibits the growth of *Proteus vulgaris* and *Bacillus cereus*. *Streptococcus aureus* and *Bacillus subtilis* have been inhibited by ethyl acetate extract at the same concentration. *Bacillus cereus* is susceptible to the petroleum ether extract at 125 µg/ml concentration.

TABLE 2A: IT SHOWS ANTIBACTERIAL ACTIVITY OF PETROLEUM ETHER EXTRACT OF *HOPE PONGA* (DENNST.), BARK WITH THE STANDARD

S. no.	Bacterial Strains	Inhibition zone in mm			
		Petroleum ether			Tetracycline
		Concentration (µg/ml)			
		250	500	1000	1
1	*Streptococcus aureus*	-	10.93±0.11	11.23±0.01	18.00±0.15
2	*Bacillus subtilis*	-	11.07±0.12	13.07±0.13	23.10±0.15
3	*Bacillus cereus*	10.07±0.12	16.97±0.03	18.00±0.10	20.07±0.12
3	*Enterobactere aerogenes*	-	10.10±0.06	11.07±0.12	15.13±0.19
4	*Proteus vulgaris*	09.00±0.12	14.87±0.09	15.97±0.15	19.07±0.07
6	*Klebsiella pneumonia*	-	17.93±0.12	19.17±0.07	28.07±0.18

TABLE 2B: IT SHOWS ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF *HOPE PONGA* (DENNST.), BARK WITH THE STANDARD

S. no.	Bacterial Strains	Inhibition zone in mm			
		Ethyl acetate			Tetracycline
		Concentration (µg/ml)			
		250	500	1000	1
1	*Streptococcus aureus*	28.00±0.15	31.23±0.19	32.93±0.13	15.00±0.17
2	*Bacillus subtilis*	21.10±0.14	27.07±0.12	31.07±0.12	20.10±0.15
3	*Bacillus cereus*	20.07±0.12	26.97±0.03	28.00±0.02	27.07±0.12
3	*Enterobactere aerogenes*	23.10±0.15	29.07±0.12	30.07±0.12	24.10±0.15
4	*Proteus vulgaris*	11.07±0.07	14.87±0.09	15.97±0.05	19.07±0.07
6	*Klebsiella pneumonia*	28.07±0.18	33.93±0.11	35.17±0.13	25.07±0.18

TABLE 2C: IT SHOWS ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *HOPEA PONGA* (DENNST.), BARK WITH THE STANDARD

S. no.	Bacterial Strains	Inhibition zone in mm			
		Methanol			Tetracycline
		Concentration (µg/ml)			
		250	500	1000	1
1	*Streptococcus aureus*	21.00±0.15	22.93±0.12	24.93±0.01	14.00±0.15
2	*Bacillus subtilis*	27.10±0.18	26.97±0.03	26.07±0.12	28.10±0.15
3	*Bacillus cereus*	20.07±0.12	27.07±0.12	28.00±0.09	27.07±0.12
3	*Enterobactere aerogenes*	20.10±0.17	21.07±0.12	22.07±0.13	19.10±0.15
4	*Proteus vulgaris*	16.07±0.07	19.87±0.09	25.97±0.15	19.07±0.07
6	*Klebsiella pneumonia*	20.07±0.18	20.83±0.12	21.17±0.12	26.07±0.18

TABLE 3: MINIMUM INHIBITORY CONCENTRATION OF *HOPEA PONGA* (DENNST.), BARK EXTRACT

S. no.	Bacterial Strains	Minimum Inhibitory Concentration (µg/ml)		
		Petroleum ether	Ethyl acetate	Methanol
1	*Streptococcus aureus*	250	100	250
2	*Bacillus subtilis*	500	125	250
3	*Bacillus cereus*	125	250	100
4	*Enterobactere aerogenes*	500	250	500
5	*Proteus vulgaris*	250	125	100
6	*Klebsiella pneumonia*	100	100	250

All the three extracts of *Hopea ponga* (Dennst.), bark *viz*., petroleum ether, ethyl acetate, and methanol showed promising antibacterial activities against almost all bacterial strains. In the present work, petroleum ether extract exhibited significant activity against *Klebsiella pneumonia* with an inhibition zone of 19.17 ± 0.12 mm. Ethyl acetate extract exhibited significant activity against *Streptococcus aureus* with an inhibition zone of 35.17 ± 0.13; methanol extract exhibited significant activity against *Bacillus cereus* with an inhibition zone of 28.00 ± 0.09 mm for 1000 mg/ml of extract respectively. The MIC values of the plant extract against the tested bacterial isolates ranged from 100 to 500 µg/ml **Table 3**. The methanol extract at the least concentration of 100 µg/ml inhibits the growth of *Proteus vulgaris* and *Bacillus cereus*. *Streptococcus aureus* and *Bacillus subtilis* have been inhibited by ethyl acetate extract at the same concentration. *Bacillus cereus* is susceptible to the petroleum ether extract at 125 µg/ml concentration.

Anthelmintic Activity: It was seen that the methanol extract of *Hopea ponga* (Dennst.), possesses dose-dependent anthelmintic activity as compared to the standard drug Albendazole, petroleum ether extract exhibited moderate activity, whereas ethyl acetate has shown less activity. The mean paralyzing time of *Pheretima posthuma* with the dose of 50 and 100 mg/ml for methanol extract was found to be 18.77 ± 0.19 and 36.50 ± 0.29 minutes respectively. Albendazole in the concentration of 25 mg/ml has taken 31.00 ± 0.0 minutes for getting paralysis. The mean death time of *Pheretima posthuma* with a dose of 50 and 100 mg/ml for methanol extract was found to be 32.97 ± 0.32 and 52.87 ± 0.41 min. In the case of Albendazole at a dose of 25 mg/ml cause paralysis only, no death was observed during the experimental period of 4 h. The results of the anthelmintic activity of different extracts are depicted in **Table 4** for petroleum ether, ethyl acetate, and methanol respectively.

TABLE 4: IT SHOWS ANTHELMINTIC ACTIVITY OF DIFFERENT EXTRACTS OF *HOPE PONGA* (DENNST.), BARK WITH THE STANDARD

S. no.	*Pheretima posthuma*	Distilled water	Petroleum ether			Ethyl acetate			Methanol			Albendazole
			Concentration of extracts (mg/ml)									
			50	100	200	50	100	200	50	100	200	25
1	Time taken for paralysis (min)	-	50.83 ±0.09	62.97 ±0.15	88.77 ±0.19	76.33 ±0.20	84.10 ±0.26	102.77 ±0.19	18.77 ±0.19	36.50 ±0.29	36.77 ±0.19	31.00 ±0.0
2	Time taken for death (min)	-	82.10 ±0.21	92.97 ±0.20	104.12 ±0.20	90.07 ±0.23	104.43± 0.26	118.43 ±0.26	32.97 ±0.32	52.87 ±0.41	62.87 ±0.41	36.00 ±0.0

***In-vitro* Antioxidant Activity:**

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity: DPPH radical is one of the few stable and commercially available organic nitrogen radicals [24]. This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH radical in test samples. DPPH radical shows a strong absorption maximum at 517 nm (purple). A freshly prepared DPPH solution exhibit a deep purple color with an absorption maximum at 517 nm. The purple color generally fades or disappears when an antioxidant is present in the medium.

Among the extracts methanol extract has shown a potent antioxidant activity at the concentration of 20 µg/ml, the extract exerted an inhibition of 51.32% and that of Ascorbic acid was 91.06%, and the IC_{50} of the extract was 19.48 µg/ml, while that of Ascorbic acid was 11.00 µg/ml **Fig. 1**. The results revealed that, dose-dependent radical scavenging activity in terms of IC_{50} values. This shows that *Hopea ponga* extract presents a good ability to scavenge the DPPH radical.

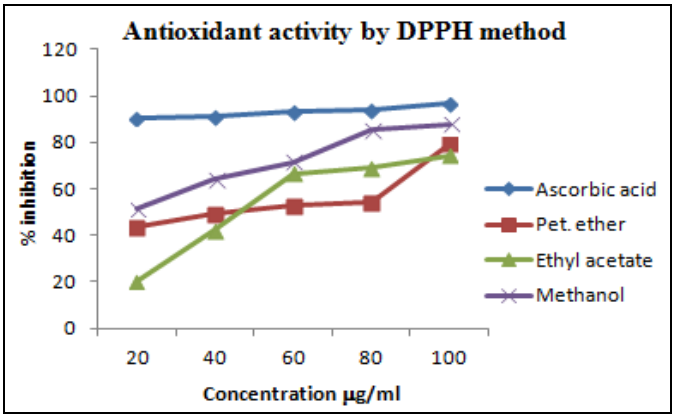

FIG: 1: IT SHOWS ANTIOXIDANT ACTIVITY OF *HOPEA PONGA* (DENNST.), BY DPPH METHOD

2, 2'-azino- bis (3- ethylbenzothiazoline- 6- sulphonic acid) (ABTS) Assay: The ABTS scavenging assay, which employs a specific absorbance (734 nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of the test samples. The *Hopea ponga extract* was found to be effective in scavenging radicals, and the increase was concentration dependent.

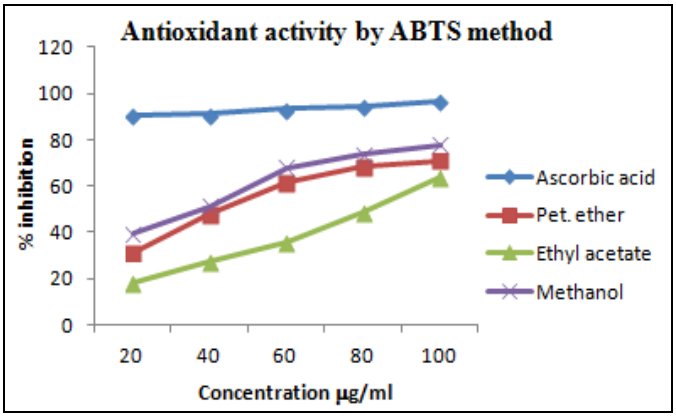

FIG. 2: IT SHOWS ANTIOXIDANT ACTIVITY OF *HOPEA PONGA* (DENNST.), BY ABTS METHOD

Among the extracts methanol extract has shown a potent antioxidant activity at the concentration of 20 µg/ml, the extract exerted an inhibition of 39.65% and that of Ascorbic acid was 91.06%, and the IC_{50} of the extract was 25.22 µg/ml, while that of Ascorbic acid was 11.00 µg/ml **Fig. 2**. The results revealed that, dose-dependent radical scavenging activity in terms of IC_{50} values. This shows that *Hopea ponga* extract presents a good ability to scavenge the ABTS radical.

Ferric Reducing Antioxidant Power (FRAP) Assay: Frap assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe^{3+}-TPTZ] complex and producing a colored ferrous tripyridyltriazine [Fe^{2+}-TPTZ]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. Frap assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction. In the present study, the trend for ferric ion reducing activities of *Hopea ponga* and ascorbic acid are shown in **Fig. 3**. The absorbance of *Hopea ponga* increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. The methanol extracts of *Hopea ponga* showed increased ferric reducing power with the increased concentration as standard antioxidants. Hence, they should be able to donate electrons to free radicals stable in the actual biological and food system.

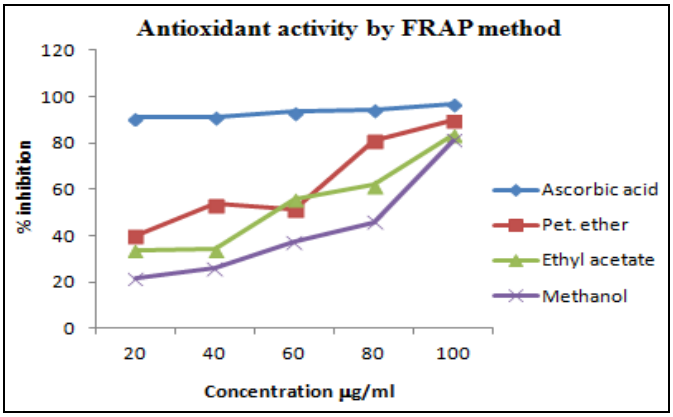

FIG. 3: IT SHOWS ANTIOXIDANT ACTIVITY OF *HOPEA PONGA* (DENNST.), BY FRAP METHOD

Among the extracts petroleum ether extract has shown a potent antioxidant activity at the concentration of 20 µg/ml, the extract exerted an inhibition of 21.39%, and that of Ascorbic acid was 91.06%, and the IC_{50} of the extract was 26.40 µg/ml, while that of Ascorbic acid was 11.00 µg/ml **Fig. 3**. The ability of the extract to reduce iron (FRAP) suggests that they contain compounds that are electron donors, which can react with free radicals to convert them to more stable products and terminate radical chain reaction.

It was reported that phenolic compounds have redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The redox potential of phenolic compounds played an essential role in determining the antioxidant potential

Hydrogen Peroxide Assay: Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once, inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects. Hydrogen peroxide scavenging activity of the extract is presented in **Fig. 4** the extract exerted concentration-dependent scavenging.

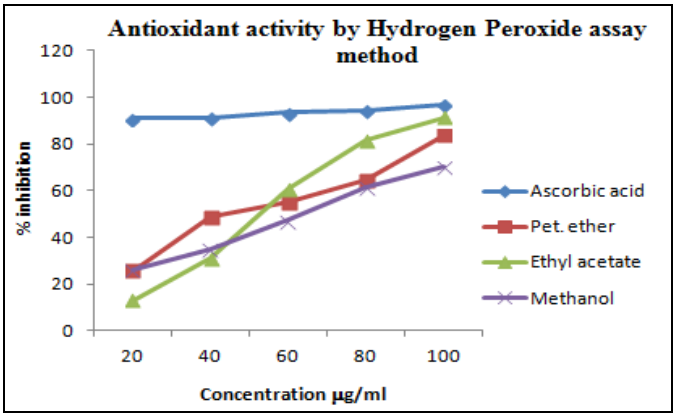

FIG. 4: IT SHOWS HYDROGEN PEROXIDE ASSAY OF *HOPEA PONGA* (DENNST.)

Among the extracts ethyl acetate extract has shown a potent Hydrogen peroxide scavenging activity at the concentration of 20 µg/ml, the extract exerted an inhibition of 26.31%, and that of Ascorbic acid was 91.06% and the IC_{50} of the extract was 38.00 µg/ml, while that of Ascorbic acid was 11.00 µg/ml **Fig. 4**. The results revealed that dose-dependent Hydrogen peroxide scavenging activity in terms of IC_{50} values.

Estimation of Total Phenolic Content: Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators [25]. Phenolic compounds are commonly found in both edible and inedible plants and have been reported to have multiple biological effects, including antioxidant activity. Phenolics can scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as the number and location of hydroxyl groups [26]. The phenolic compounds such a phenolic acid and flavonoids are the most important antioxidant food source. The quantitative analysis of phenolic acids and flavonoids by the measurement of UV absorption is well known [27]. In the present study, the total phenolic content of *Hopea ponga* (Dennst.), bark extract was analyzed. The total phenolic content was determined using Folin-Ciocalteu's method and total phenolic content of the extract was expressed in terms of milligrams of Catechol per gram of dry weight by reference to standard curve **Fig. 5**. ($Y = 0.207x + 0.444$ and $R^2 = 0.978$).

Total phenolic content in different extracts of the plant used in the present study is presented in **Table 5**. It is clear that the level of polyphenols in the methanolic extract of *hope ponga* (Dennst.), was higher when compared to ethyl acetate and pet ether extract.

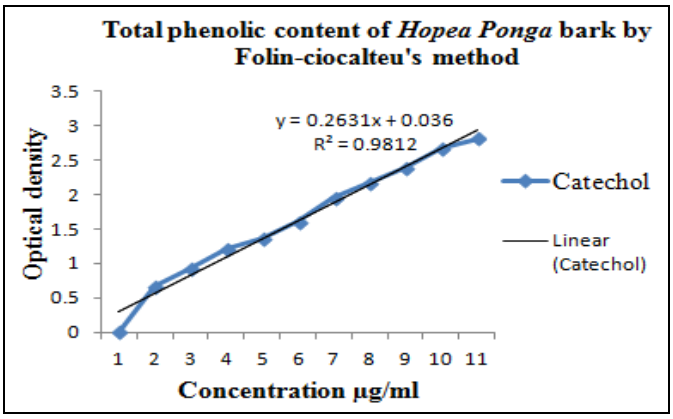

FIG. 5: IT SHOWS THE TOTAL PHENOLIC CONTENT OF *HOPEA PONGA* (DENNST.), BARK WITH THE STANDARD

TABLE 5: IT SHOWS THE TOTAL PHENOLIC CONTENT OF *HOPEA PONGA* (DENNST.)

Extracts	Total phenolic content (mg of Catechol equivalents/100mg dried extract)
Petroleum ether	135.15±1.25
Ethyl acetate	160.10±1.65
Methanol	195.11±2.14

Medicinal plants contain various phytochemical compounds that attribute to their medicinal properties. A perusal of the literature reveals that the majority of the antioxidant activity is due to the polyphenols, flavones, isoflavones, flavonoids, anthocyanin, coumarins, lignans, catechins and isocatechins [28]. The medicinal value of polyphenols in the plants is due to their higher antioxidant nature. Phenolic compounds are a class of antioxidant agents, which act as free radical terminators [29]. The preliminary phytochemical analysis of the pet ether, ethyl acetate and methanol extract of bark showed the presence of alkaloids, flavonoids, phenolics, steroids, saponins, terpenoids and tannins this may account for the antioxidant potential of the extracts. The free radical scavenging activity of the plant extract contributes to the neutralization of free radicals, thereby inhibiting chain reaction and stops cellular damage within body cells.

Hence, *in-vitro* antioxidant activity was determined by the DPPH radical scavenging method and total phenolic content assay. The results confirmed that the petroleum ether extract exhibited potent

antioxidant activity in comparison to that of ethyl acetate and methanol extracts. Among the extracts, petroleum ether extract has shown a potent hydrogen peroxide assay antioxidant activity. Among the extracts, methanol extract has shown a potent hydrogen peroxide assay antioxidant activity. The similar activity was 11.00 µg/ml for standard ascorbic acid **Fig. 5**.

It was found that the minimum is the value of IC_{50}, the maximum is the antioxidant activity. The results revealed that, dose-dependent radical scavenging activity in terms of IC_{50} values. Total phenolic content in methanol extract was found to be 195.11 ± 2.14 followed by ethyl acetate extract having 160.10 ± 1.65 and pet ether extract having 135.15 ± 1.25 expressed as mg of catechol equivalents/100mg dried extract. The results of the two procedures are correlated to each other and confirmed the use of the plant as a natural antioxidant.

CONCLUSION: The present study reveals that the crude drug posse's prominent antimicrobial, anthelmintic and antioxidant properties, which supports its folk claim. Phytochemical studies portray the presence of several biologically active secondary metabolites. Therefore, there is no doubt that this plant is a reservoir of potentially useful chemical compounds, which serve as drugs, provide newer leads and clues for modern drug design.

ACKNOWLEDGEMENT: We are grateful to the Department of Chemistry, Sahyadri Science College, Shivamogga, for providing facilities to carry out the present research work in a soothing manner. It's also our pleasure to thank the Directorate of Minorities, Bengaluru, for awarding the fellowship, through the Ph.D. fellowship.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interests.

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How to cite this article:
Ajish AD, Vagdevi HM and Sunitha BJ: Evaluation of antimicrobial, anthelmintic and antioxidant activities of *Hopea ponga* (Dennst.) bark. Int J Pharm Sci & Res 2019; 10(4): 1838-47. doi: 10.13040/IJPSR.0975-8232.10(4).1838-47.