measured from the bleaching of the purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2, 2- diphenyl-1-picrylhydrazyl (DPPH), as a reagent. The working solution of the test extract was prepared in methanol. BHT and Gallic acid were used as standard in 1-100µg/ml solution. DPPH (0.002%) was prepared in methanol and 1ml of sample solution separately. The solution mixture was kept in dark for 30min and absorbance was measured at 517nm. DPPH solution (1ml) was used as blank. The absorbance was recorded and % inhibition was calculated using the formula given below.

$$\% \text{ scavenging activity} = \frac{A-B}{A} \times 100$$

Where A= absorbance of the control and
B= absorbance of the sample
IC_{50}= value, which represented, as the concentration of methanolic extract that caused 50% scavenging as calculated from the plot inhibition percentage against concentration.

2.4.2 Nitric Oxide scavenging method

The Nitric Oxide scavenging method elucidated by **(10)** was followed**.** Nitric oxide (NO) was generated from Sodium Nitroprusside (SNP) and was measured by Griess reagent. SNP in aqueous solution at physiological pH spontaneously generate NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavenging of NO compete with oxygen leading to reduced production of NO. SNP (10mM) in phosphate buffer saline (PBS) was mixed with different concentration of extract (100-1000µg/ml) of the drug dissolved in methanol and incubated at 25^0C for 180 min. The samples from the above were reacted with Griess reagent (1% sulphanilic acid, 0.1% naphthylamine and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilic acid and subsequent coupling with napthylamine was recorded at 546 nm and referred to the absorbance of Gallic acid, used as a positive control treated in the same way with Griess reagent.

2.4.3 Antibacterial activity

Antibiogram was done by agar well diffusion method **(11, 12)** using plant extracts and commonly used antibiotics. The test quantity of specific extracts were dissolved in dimethylsulphoxide (DMSO), depending upon the solubility of the extracts. The dissolution of the organic extracts was aided by 1% (v/v) DMSO, which did not affect the growth of microorganisms, in accordance with our control experiments. The surface of media was inoculated with bacteria from a broth culture. Wells or cups of 5 mm size were made with sterile borer into agar plates containing the bacterial inoculums. 20µl volume of the sample extracts of concentration (2.0 mg/ ml) was poured into a well of inoculated plates. Ampicillin (10mg/ml) was used as a positive control which was introduced into the well instead of plant extract. After 18 h of incubation at a specific temperature the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter. Antibacterial activity was recorded if the radius of zone of inhibition was greater than 4 mm **(13)**. The antibacterial activity results was considered as inactive if < 4.5 mm; 4.5-6 mm as partially active; while 6.5-9 mm as active and greater than 9mm as very active **(14)**.

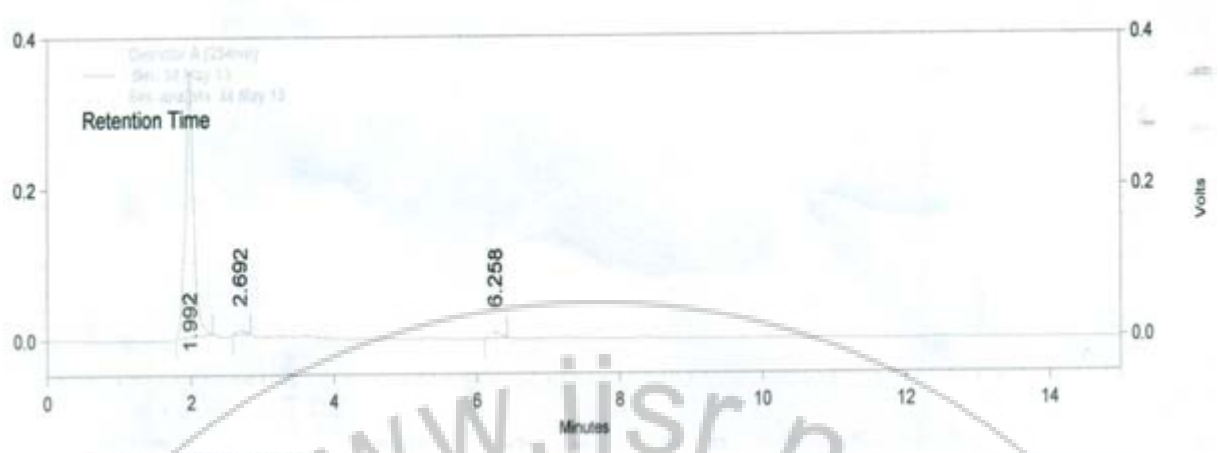
2.5 Minimum Inhibitory Concentration assay

Minimum Inhibitory Concentration of fruit peel extracts was also determined **(15, 16)**. Dilution of the fruit peel extracts of Citrus Karna was prepared in sterile nutrient broth to achieve a decreasing concentration ranging from 1000µg/ml to 12µg/ml in sterile tubes labeled 1 to 5. Each dilution was seeded with 10µl of the standardized bacterial inoculums (108-109) CFU/ml. The inoculated culture tubes were incubated at 37°C for 24hrs. A set of tubes containing only seed broth (i.e. without plant extract) was kept as control. The lowest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC value of the extract. After incubation, 10µl of content of each test tube was transferred with a loop on to nutrient agar media. Agar plates were incubated for 24 hours at 370C. The lower concentration that did not permit any visible growth when compared with the control was considered as the MIC.

3. Results and Discussion

3.1 HPLC of the methanol extract of Berberis aristata

HPLC fingerprinting was established with good repeatability. Following three peaks have been shown in spectra .The compounds have been identified by comparing with the standards and also by comparing with the data reported for the *Berberis aristata* in literature. The identification has been done on the basis of Retention time and percentage height. *Berberis aristata* contains alkaloids which are berbamine, berberine, oxycanthine, epiberberine, palmatine, dehydrocaroline, jatrorhizine and columbamine **(17).** The major alkaloid found in *B. aristata* is Berberine followed by Palamatine. While analyzing the components present in methanol extract of *Berberis aristata* through HPLC the three components which were identified are Berberine of 95.52%, Palmitine of 2.64% and Jatrorrhizine of 1.83% **(Figure 1).**



Detector A (254nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.992	2729742	95.527	351364	96.344
2	2.692	52337	1.832	5516	1.512
3	6.258	75489	2.642	7819	2.144
Totals		2857568	100.000	364699	100.000

Figure 1: Spectrum for the HPLC of methanolic extract of *Berberis aristata.*

3.2 Antioxidant Activities

The DPPH free radical scavenging activity of methanolic extract of *Berberis aristata* at various concentrations was determined and compared with that of the standard BHT and Gallic acid **(Table 2 and Figure 2).** The methanolic extract reduced the concentration of DPPH free radical with efficiency near to that of Gallic acid but less than BHT. The IC_{50} value for methanolic extract was 0.90mg/ml.

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by Griess Reaction. Nitric oxide radical generated from nitroprusside at physiological pH was found to be inhibited by the methanolic extract as shown in the (**Table 3 and Figure 3)**.

The IC_{50} values have been found to be 0.80 mg/ml, 0.65 mg/ml and 0.60mg/ml for the methanolic extract, gallic acid and BHT respectively.

Table 2: DPPH free radical scavenging assay (%) of methanolic extract of *Berberis aristata*, Gallic acid and BHT.

Conc.(mg/ml)	Extract	%	Gallic acid	%	BHT	%
1.0	0.321±0.006	58.31	0.383±0.011	58.45	0.234±0.018	66.42
0.8	0.447±0.017	41.94	0.410±0.006	55.53	0.265±0.006	61.97
0.6	0.535±0.028	30.51	0.470±0.007	49.02	0.332±0.011	52.36
0.4	0.640±0.046	16.88	0.532±0.028	42.29	0.365±0.007	47.63
0.2	0.660±0.054	14.28	0.554±0.037	39.91	0.445±0.042	36.15

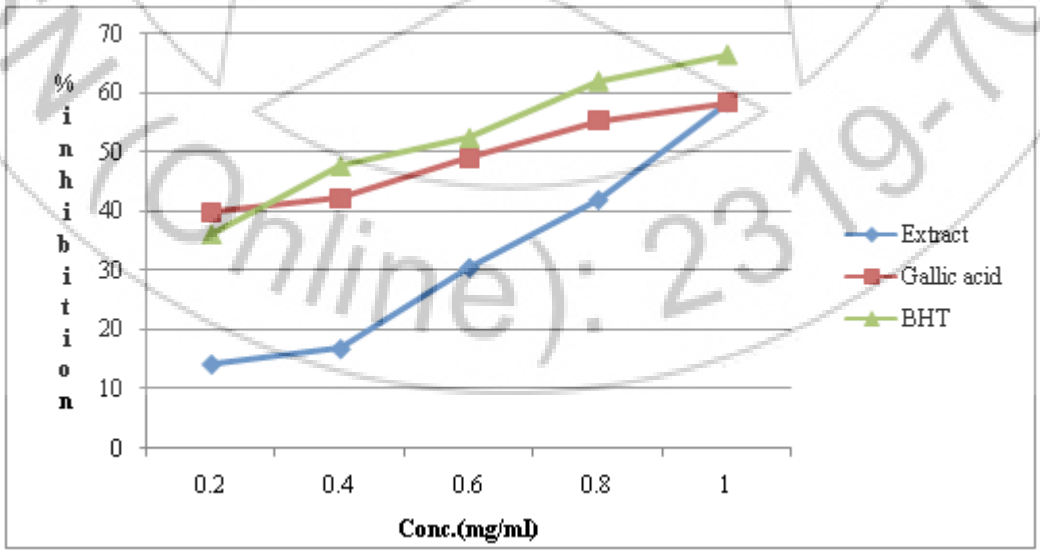


Figure 2: DPPH scavenging activity for BHT, Gallic acid and methanol extract of *Berberis aristata.*

Table 3: Nitric Oxide Scavenging method (%) of methanolic extract of *Berberis aristata*, Gallic acid and BHT.

Conc.	*Extract*	*%*	*Gallic acid*	*%*	*BHT*	*%*
1.0	0.517±0.016	54.32	0.539±0.010	53.67	0.698±0.060	56.51
0.8	0.569±0.045	49.73	0.552±0.010	51.74	0.758±0.036	52.77
0.6	0.606±0.072	46.46	0.579±0.005	49.38	0.806±0.016	49.78
0.4	0.734±0.052	35.15	0.633±0.008	44.66	0.837±0.023	47.85
0.2	0.813±0.060	28.18	0.684±0.010	40.20	0.868±0.013	45.91

Values were expressed as MEAN ± S.D. (n=3)

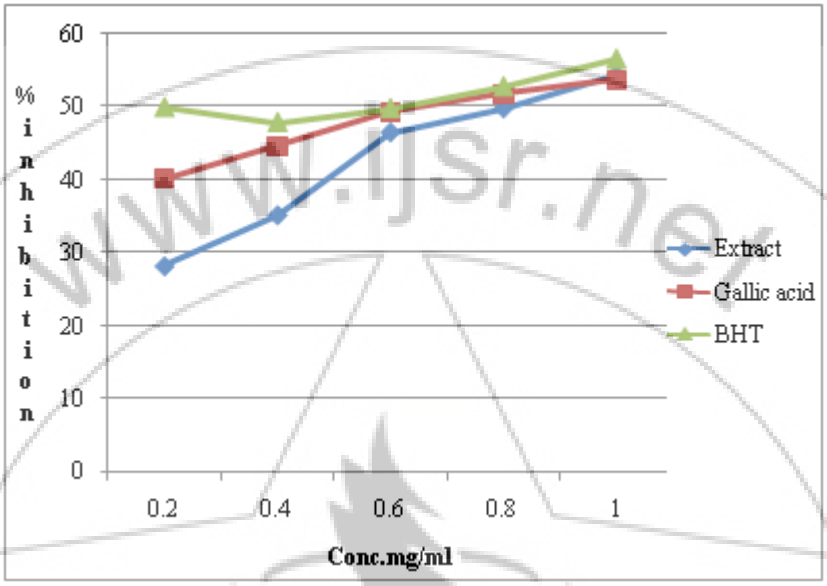
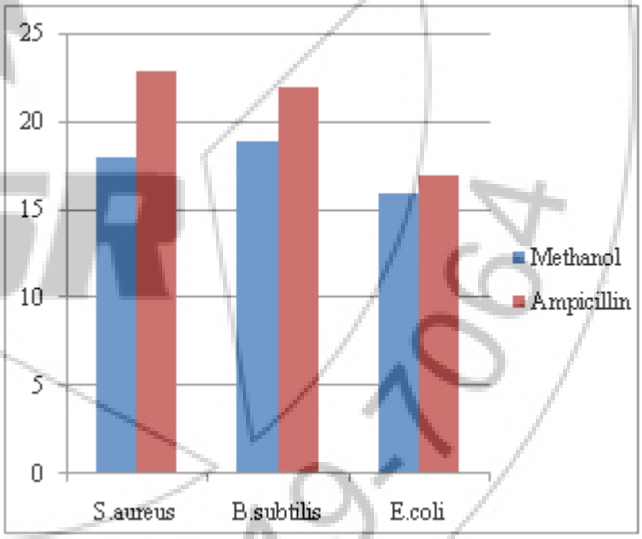


Figure 3: Ntric Oxide scavenging activity for BHT, Gallic acid and methanolic extract of *Berberis aristata.*

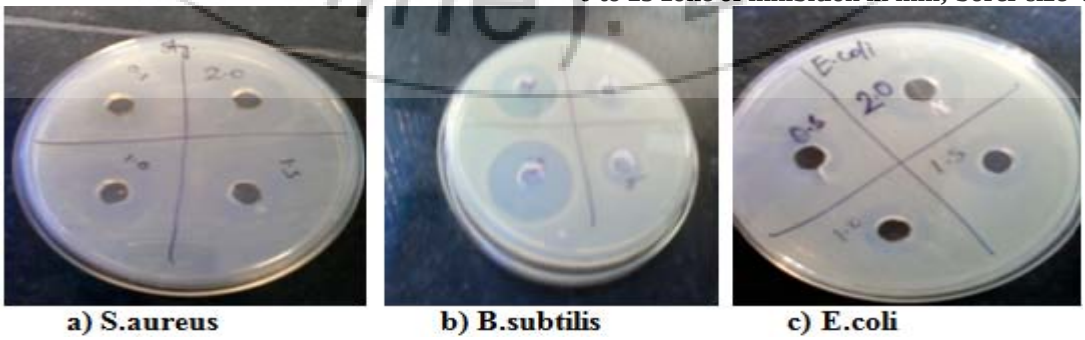
3.3 Antibacterial activity

The antibacterial activity reveals that the methanolic extract of *Berberis aristrata* stem is highly active against both Gram positive and Gram negative bacteria. The methanolic extract shows highest zone of inhibition (19mm) against *B.subtilis* followed by (18mm) zone of inhibition against *S.aureus* and lowest (16mm) zone of inhibition against *E.coli*. Graph 1 summarizes the microbial growth of methanolic extract and ampicillin used as a positive control.



Graph 1: Antibacterial activity (in mm) of methanolic extract and ampicillin (standard)

***0 to 25 zone of inhibition in mm; borer size=5mm**



a) S.aureus **b) B.subtilis** **c) E.coli**

Figure 4: Antibacterial activity of *Berberis aristata* extract by agar well diffusion.

a) S.aureus b) B.subtilis c) E.coli

Figure 5: Antibacterial activity of Ampicillin

The MIC which is lowest concentration of a plant extract that still retained an inhibitory effect against the growth of a microorganism was assessed by using broth dilution method. The MIC values of methanol extract of *Berberis aristata* for different pathogenic bacteria ranged from (37 to 111) µg/ml.

Table 5: Intial MIC of *Berberis aristata* methanolic extract against bacteria

Conc.(µg/ml)	*Berberis aristata* methanol extract (µg/ml)against bacteria		
	S.aureus	*B.subtilis*	*E.coli*
1000	-	-	-
333	-	-	-
111	-	*	*
37	*	+	+
12	+	+	+
MIC	37	111	111

(+)Growth, (*)MIC and (-)No growth

4. Conclusion

On the basis of results obtained it can be concluded that *Berberis aristata* stem possess alkaloids and have potent antioxidant and antibacterial activities. Further the potential of this plant can be explored more and more, in order to develop an alternative therapy for treatment of various diseases. The present study also suggests that the use of this medicinal plant may be exploited for health supplements. Thus justifying its traditional use.

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