

## Antimicrobial Activities of Extract from Seeds of *Baccaurea Ramiflora*

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**Abstract:** *Baccaurea ramiflora* is a source of vast variety of compound valuable for pharmaceutical industry. It was designed to examine chemical and microbiological investigation of the plant *Baccaurea ramiflora* to identify its chemical constituents and to elucidate the antimicrobial activity. It was analyzed by Thin layer Chromatography (TLC). For microbial investigation, crude extracts of the seeds of the plant with pet-ether, chloroform, ethyl acetate and methanol were screened against three highly pathogenic organism i.e. *Escherichia coli*, *Staphylococcus aureus* and *Shigella sonnei*. All the extracts except petroleum ether the ethyl acetate, chloroform and methanol extracts were very effective in inhibiting the growth of *Escherichia coli*. Chloroform extract was taken for determine the chemical constituent in the present studies as it displayed strongly inhibitory effect on antibacterial activity. Finally, this review will also provide future research direction for the essential oil and extracts of *Baccaurea ramiflora* could serve as an important bio-resource of antioxidants for using the pharmaceutical industries.

**Keywords:** *Baccaurea ramiflora*, Antioxidant activity, Antimicrobial activity, Essential oil.

### 1. INTRODUCTION

The chemistry of natural product is both fundamental and complex. In nature plants of several variations are available which are responsible for various pharmacological actions. They are termed as medicinal plants. Antioxidants are vital substances having the ability to protect the body from damage caused by free radical induced oxidative stress. Synthetic antioxidants have restricted use in foods as they are suspected to be carcinogenic<sup>1</sup>. Therefore, the importance of searching natural antioxidants has greatly increased in recent years<sup>2,3</sup>. Modification, improvements, sophistication's and newer discoveries are continuously changing the type, quality of medicinal preparations. However, plant-derived drugs still feature in modern medicine can be assessed. A recent survey by the United Nations Commission for Trade and Development (UNCTAD) indicated that about 33% drugs produced in the developed countries, are derived from plants and that if microbes are added 60% of medicinal products are of natural origin<sup>4</sup>. According to some sources almost 80% of present day medicines are directly or indirectly derived from plants. The consumption of medicinal plants is increasing in many developed countries, where 35% of drugs contain active principle from natural origin<sup>5,6</sup>.

*Baccaurea ramiflora*, the Burmese grape is a slow growing evergreen tree in the Phyllanthaceae family, growing to 25m. It is found throughout Asia, most commonly cultivated in India, Bangladesh and Malaysia. It grows in evergreen forests on a wide range of soils. The fruit is harvested and used locally, eaten as a fruit, stewed or made into wine; it is also used medicinally to treat skin diseases. The bark, roots and wood are harvested for medicinal uses. The fruit is oval, colored yellowish, pinkish to bright red or purple, 2.5–3.5 cm in diameter, glabrous, with 2–4 large purple-red seed, with white aril. It is a plant that is used in the treatment of many diseases like asthma, bronchitis, fever, piles, pain in abdomen, at the anus and in the muscles.

### 2. MATERIAL AND METHODS

#### 2.1. Plant Material

The plant *Baccaurea ramiflora* was collected from Shalikh, Magura, Bangladesh. Before collection of plant was taxonomically identified by the Bangladesh National Herbarium at Dhaka. The plant was

cleaned and cut into small pieces and then dried under shade. When the plant was properly dried they were pulverized into a coarse powder by grinding mill.

## **2.2. Isolation of Essential Oil and Preparation of Organic Extracts**

The powder 245gm soaked in distilled petroleum ether at room temperature for 7 days in a large conical flask. The mixture was stirred by glass rod every day an hour. After 7 days the pet-ether extract was filtered through a filter paper in several times. The residual plant material was extracted successively with chloroform, ethyl acetate and finally with methanol.

The eight extracts i.e petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf, root and stem of the plant were filtered. These were performed by passing the extracts through filter paper. After filtration each of the extract was concentrated to dryness in a rotary evaporator at 40 °c under vacuum.

Thin Layer chromatography (TLC) is the most helpful method for the detection of organic compounds in a mixture. TLC was carried out on glass plates as 5x20 cm<sup>2</sup>. The glass plates were cleaned with Na<sub>2</sub>CO<sub>3</sub> solution to remove greasy substances, washed with water and acetone, dried and placed on a thin layer chromatography template. The plates were tightened with the level. 24gm of silica gel were taken into a conical flask, 48ml of water added and shaken well to make slurry. It was allowed to rest for a minute to float bubbles and then poured into the spreader set at 0.25mm thickness. The spreader slotted through the slurry was traveled over the glass plates and thus a layer was deposited on the plates. The plates then allowed staying in horizontal position at room temperature for two hours for drying and were finally activated by heating in an oven (100 °C) for 2hrs. Applied on the activated TLC plates with capillary tube. Care was taken during development that potted line. The dried plates were treated with suitable reagent as iodine vapor and Spray reagents (vanillin sulfuric acid and Dragendroff's reagent). The chloroform extract (2.8g) was greenish gummy substance, almost insoluble in pet-ether, sparingly soluble in ethyl acetate and dichloromethane but readily soluble in methanol and chloroform. The TLC examination showed that resolution was best in 50% dichloromethane in ethyl acetate with tailing from base line.

The extract was dissolved in small volume of CHCl<sub>3</sub> and was adsorbed on a small quantity of silica gel. It was then completely dried under reduced pressure on a rotary evaporator at temperature 40°C and very carefully poured on the top of a silica gel column mode of solid silica gel in Buchner funnel. The column was the eluted successively with pet-ether, dichloromethane and different mixtures of ethyl acetate and dichloromethane. it shows when eluting solvent is 100% PE then TLC examination shows Two sports with R<sub>f</sub> values 0.57, 0.49 with long tailing and the observation is Mixtures of two compounds with long tailing which Yield in 0.002 gm. Similarly eluting solvent is 100% EA then TLC examination shows Three sports with R<sub>f</sub> values 0.87, 0.79, 0.68 with observation Mixtures of three compounds and yield in 0.005gm. TLC examination of this crystal showed single spot on TLC plates having R<sub>f</sub> values 0.53 in 30% ethyl acetate in pet-ether. The crystal was again dissolved in minimum amount of chloroform under boiling condition and allowed to stand overnight at room temperature. A white needle shaped crystal was separated out. TLC examination of this crystal showed single spot on TLC plates having R<sub>f</sub> values 0.53 in 30% ethyl acetate in pet-ether.

## **2.3. Antimicrobial Activity**

*DPPH(2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay:*

The DPPH scavenging activity of the plant extracts was measured according to producer by Blois<sup>7</sup> and Desmarcheliet al.<sup>8</sup> with mirror modification. 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.02mM) was prepared by dissolving 7.886g of DPPH in 100ml of methanol. 20 ul of plant extract with DMSO or standard (ascorbic acid in methanol) were added with 180 ul of DPPH solution. The reaction was incubated for 30 minutes at room temperature in dark. After incubation, the absorbance was read at 517 nm using microplate reader. The percentage of radical scavenging activity (RSA) was calculated using equation:

$$\%RSA = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) * 100}{(\text{Absorbance of control})}$$

## 2.4. Total Phenolic Content

TPC of the plant was determined based on the Folin-Ciocalteu colorimetry assay method as established by Waterhouse [8] with slight modification. 50  $\mu$ l of Folin-Ciocalteu's phenol reagent was added with 10  $\mu$ l of plant sample of different concentration or gallic acid standard. After 5 minutes, 100  $\mu$ l of sodium carbonate was added and mixed thoroughly by re-suspending using a micropipette and was incubated for 90 minutes at room temperature. Next, the absorbance of the sample and standard were measured at 760 nm with a microplate reader. The TPC in leaf extracts were calculated using equation (2). (GAE, is the Gallic Acid Equivalence (mg/ml), V, is the volume of extract (ml), M = the weight of pure plant extract (g).

## 2.5. Statistical Analysis

Three organisms such as *Escherichia coli*, *Shigella Sonnei*, and *Staphylococcus aureus* were tested to determine the antibacterial effect of crude extract from the examined plant. The experiment was replicated two times and the data were analyzed using mixed procedure of the statistical analysis software to determine significant differences in the antibacterial effects of *Baccaurea ramiflora*. The Antibacterial activities were determined by measuring the diameter of the zone in mm.

## 3. RESULT

### 3.1. Antioxidant Results

**Table 1.** Total phenolic compounds of *Baccaurea ramiflora* extracts

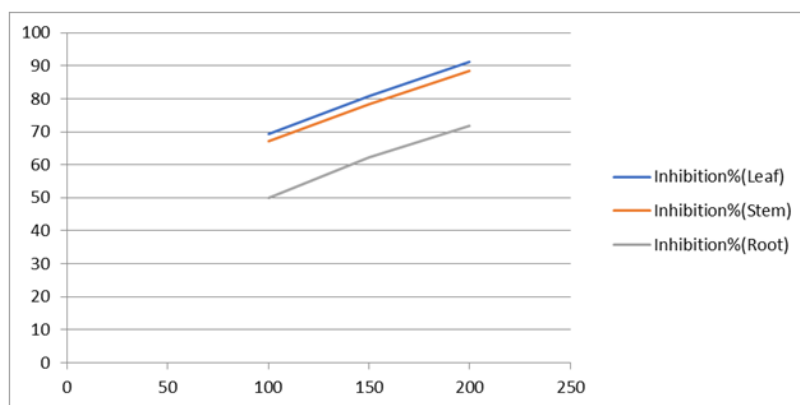
Extracts	Total phenolic(mg GAE/g dw) <sup>a</sup>
Methanol extract	71.63 $\pm$ 2.2
Hexane fraction	24.87 $\pm$ 1.8
Chloroform fraction	39.43 $\pm$ 0.8

<sup>a</sup> Values are given as the mean  $\pm$  S.D. of triplicate experiments.

Table 1 shows the total phenolic of DPPH scavenging activity among crude extracts of *Baccaurea ramiflora*. The *Baccaurea ramiflora*(BR) shows the higher followed by Methanol (71.63mg GAE/g dw)<sup>a</sup>. The Hexane fraction of BR reach lowest concentration as (24.87mg GAE/g dw)<sup>a</sup> of crude extract. Where Chloroform fraction shows higher than Hexane fraction as (39.43 mg GAE/g dw)<sup>a</sup>.

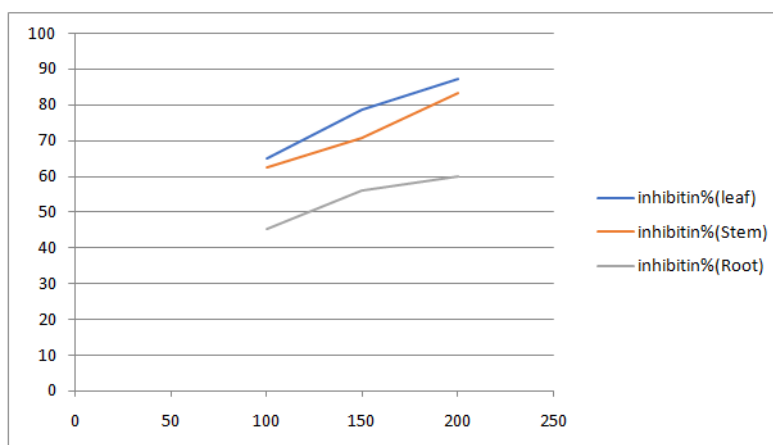
**Table 2.** DPPH scavenging activities of *Baccaurea ramiflora* in methanolic and ethanolic extract

Extract	Used part	Conc. $\mu$ g/ml	Inhibition%	IC <sub>50</sub> Value
Methanol Extract	Leaf	100	71.56 $\pm$ 1.2	10.35
		150	85.21 $\pm$ 1.5	
		200	96.12 $\pm$ 1.1	
	Stem	100	69.38 $\pm$ 1.2	15.27
		150	80.47 $\pm$ 1.3	
		200	92.14 $\pm$ 1.4	
	Root	100	55.15 $\pm$ 1.5	72.24
		150	69.67 $\pm$ 0.5	
		200	77.64 $\pm$ 1.5	
Ethanol Extract	Leaf	100	67.23 $\pm$ 1.7	22.41
		150	81.45 $\pm$ 1.6	
		200	90.54 $\pm$ 1.4	
	Stem	100	65.14 $\pm$ 1.3	36.05
		150	81.15 $\pm$ 1.1	
		200	90.54 $\pm$ 0.5	
	Root	100	48.12 $\pm$ 0.7	104.43
		150	61.11 $\pm$ 1.2	
		200	68.14 $\pm$ 1.2	



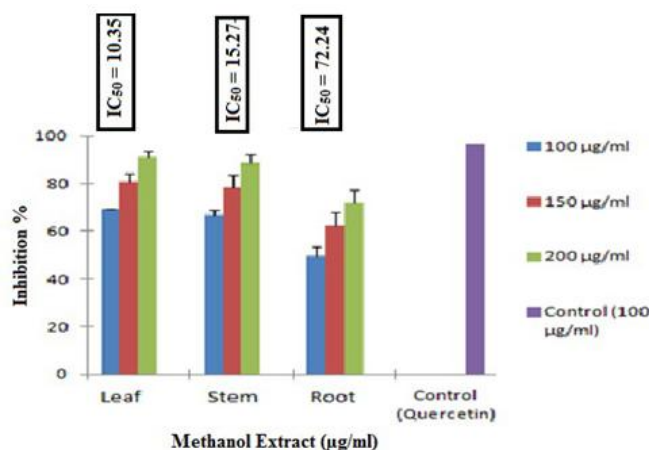
**Fig1.** DPPH Scavenging activities of *Baccaurea ramiflora* in methanolic extract

This line graph shows a uniform increase in the DPPH scavenging activity of *BR* leaf extract as the concentration of crude extract increases. The ME of *BR* shows the highest DPPH scavenging activity of leaf in between (71.56 – 96.12) % inhibition followed by ME and the lowest value of DPPH scavenging activity of root in between (55.15-77.64)% inhibition. The level shows of stem in (69.38-92.14)% inhibition. The differences in DPPH scavenging activity % among the crude extracts are significant.



**Fig2.** DPPH Scavenging activities of *Baccaurea ramiflora* in ethanolic extract

This line graph shows a uniform increase in the DPPH scavenging activity of *BR* leaf extract as the concentration of crude extract increases. The ethanolic extract of *BR* shows the highest DPPH scavenging activity of leaf in between (67.23 – 90.54) % inhibition followed by ethanolic and the lowest value of DPPH scavenging activity of root in between (48.12-64.14)% inhibition. The level shows of stem in (65.14-90.54)% inhibition. The differences in DPPH scavenging activity % among the crude extracts are significant.



**Fig3.** DPPH Scavenging activities of *Baccaurea ramiflora* in methanolic extracts

Fig.4 shows all *BR* leaf crude extract possesses the ability with methanol in different efficiency. The ME of *BR* root shows highest extraction in 200Conc.µg/ml and the IC<sub>50</sub> Value is 72.24 and the Lowest extraction observed leaf in 10.35 IC<sub>50</sub> Value. where the middle IC<sub>50</sub> shows 15.27.

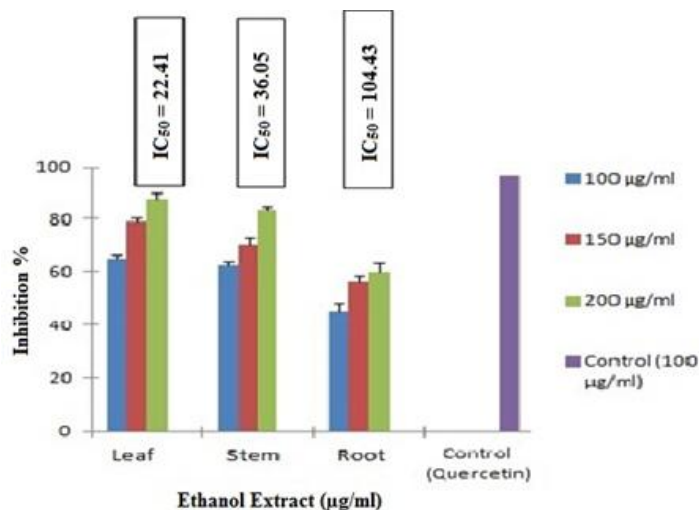


Fig4. DPPH Scavenging activities of *Baccaurea ramiflora* in ethanolic extracts

Fig.4 shows all *BR* different parts crude extract possesses the ability with methanol in different efficiency. The ethanolic of *BR* root shows highest extraction in 100Conc.µg/ml and the IC<sub>50</sub> Value is 104.43 and the Lowest extraction observed leaf in 22.41 IC<sub>50</sub> Value. Where the middle IC<sub>50</sub> shows 36.05.

Table3. Antibacterial activity and minimum Inhibitory Concentration (MIC) values of chloroform extract of stem of *Baccaurea ramiflora*

Test of the organism	Concentration of the disc (µg/ml) and obtained zone of inhibition (mm)										Positive Control µg/ml
	512 µg/ml	256 µg/ml	128 µg/ml	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	2 µg/ml	Negative control µg/ml	
Escherichia coli	10 mm	8 mm	+	7 mm	+	+	+	+	+	+	15 Mm
Staphylococcus aureus	8 mm	+	+	+	+	+	+	+	+	+	12 Mm
ShigellaSonnei	+	+	+	+	+	+	+	+	+	+	+

+ = Growth

### 3.2. Chloroform Extract of Strem of *Baccaurea ramiflora* on Escherichia Coli

Chloroform extract of stem of *Baccaurea ramiflora* produced zone of inhibition against Escherichia coli. The diameters of zone on inhibition were found 10mm, 8mm and 7mm for the concentrations of 512µgml<sup>-1</sup>, 256µgml<sup>-1</sup> and 64µgml<sup>-1</sup> respectively. The Staphylococcus aureus produce 8mm against of 512µgml<sup>-1</sup>.

Petroleum ether extract does not show any antibacterial activity against all the tested bacteria. Generally low polar substances such as oils, fats, waxes, hydrocarbons, glycerodies are washed by petroleum ether (b.p 40-60 °C) solvent. This may be the reason for the low or no bactericidal activity of petroleum ether extract in the present study.

## 4. DISCUSSION

All the extracts except petroleum ether showed a significant antibacterial activity against almost all the tested bacteria. Ethyl acetate, chloroform, methanol extract was very effective inhibiting the growth of Escherichia coli. Ethyl acetate and chloroform extract of *Baccaurea ramiflora* was also

very effective in inhibiting the growth of *ShigellaSonnei* and *Staphylococcus aureus*. The extracts of *Baccaurea ramiflora* have been reported to possess antibacterial activity.

## 5. CONCLUSION

This study concludes that the antioxidant and radical scavenging activity of the essential oil and organic extracts of *Baccaurea ramiflora* leaves indicate towards its strong protective role against oxidative diseases and possible use of *B. ramiflora* leaves as a natural antioxidant for potential pharmaceutical application. The oil showed higher antioxidant activities than organic extracts, whereas methanol extracts as compared to three tested methods. Further work is needed to fully understand the variables that can affect the evaluation of the antioxidant capacity by different methodologies.

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