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# Phytochemical Investigation and Biological Studies of *Coffea benghalensis* B. Heyne Ex Schult

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**Abstract**

The qualitative phytochemical screening of leaves and stems of *Coffea benghalensis* B. Heyne Ex Schult showed the presence of saponins, flavonoids, steroids, and cardiac glycoside. Stigmasterol, 1, 3- stearyl-oleyl-glyceride and stigmasterol glucoside were isolated and characterized from the dichloromethane extract of this plant leaves and stems by chromatographic and spectroscopic methods. Total caffeine content per hundred grams was estimated. Fatty acids were analyzed quantitatively by GLC. Total antioxidant capacity and antimicrobial activity of the plant leaves and stems extract were studied.

**Keywords:** *Coffea benghalensis*, caffeine; stigmasterol; 1, 3-stearyl-oleoyl-glyceride; fatty acid.

## I. Introduction

Extracts procured from *Coffea* plants have long been acknowledged for their robustness, antioxidant capacity[1] and immunomodulation[2]. The plant *Coffea benghalensis* B. Heyne Ex Schult is used as folk medicine in Bangladesh as well as in Indian subcontinent. Low concentration of caffeine was reported by HPLC analysis of the aqueous extracts of endosperm from mature and immature fruits of *Coffea benghalensis* B. Heyne Ex Schult[3]. Caffeic acid was dominant in the hydrolysed extract whereas chlorogenic acid in the non-hydrolysed seed extract, and isoquercitrin and rutin in the non-hydrolysed extract of the leaf of this plant[4]. Caffeine occurs naturally in leaves, seeds and fruit of several plants and trees. Caffeine belongs to xanthine alkaloid which acts as a natural insecticide[5]. It is a vital constituent of tea, coffee and chocolate which acts as a central nervous system (CNS) stimulant in humans[5].These are extremely bitter in taste[5]. Despite of lacking scientific proof in assuring the enough phytochemical and antimicrobial activity of the plant *Coffea benghalensis* B. Heyne Ex Schult, it is frequently availed by the societies for medicament of several ailments. So, the motive of this study was to estimate caffeine content in the seed, to screen the significant phytochemical components and to evaluate the biological potentialities of the leaf and stem extracts of the plant to carry the traditional sanative claim and to impart standard information for the scientific communities to carry on further study.

## II. Experimental

### *Reagents and solvents*

Laboratory or analytical grade reagents and solvents utilized during the research were procured from BDH (England) and Merck (Germany). Before use, the commercial grade solvents were distilled.

### *Collection of sample and preparation*

The whole plant *Coffea benghalensis* B. Heyne Ex Schult (Locally known as Bonnya Kafee, Bengal Coffee) were collected from Savar, Dhaka. In the Bangladesh National Herbarium (BNH), a voucher specimen of this plant was deposited having DACB ACCESSION NO 38728. The dried leaves and stems were separately grinded to powder by a Cyclotec grinder (200 meshes) which was used throughout the investigation.

### *Determination of caffeine content*

Dried seeds powder was extracted with water and the extract was collected and dried. Caffeine stock solution was prepared by dissolving 10 mg of caffeine in dichloromethane. A calibration curve of UV spectrum was prepared by using caffeine standard solutions. The absorbance of the aqueous solution of the sample was measured from calibration curve[6].

### *Extraction*

The dried leaves powder and stems powder were extricated individually with petroleum ether (b.p. 40-60°C), subsequently dichloromethane (DCM) and methanol successively.

### *Isolation of compounds from dichloromethane extract*

The dichloromethane extract of *Coffea benghalensis* B. Heyne Ex Schult leaves was subjected to column chromatography and the two fractions $\mathbf{F_3}$ and $\mathbf{F_8}$ were appeared to contain single compound having the different and distinct $R_f$ values. Further purification of each of the two fractions was carried out separately by pipette column. The fractions $\mathbf{F_3}$ and $\mathbf{F_8}$ were named **A** and **B**, respectively.

Again the dichloromethane extract of *Coffea benghalensis* B. Heyne Ex Schult stems was subjected to column

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chromatography and two fractions $\mathbf{M_1}$ and $\mathbf{M_2}$ were appeared to contain single compound having the distinct $R_f$ values. After purification, the fraction $\mathbf{M_1}$ and $\mathbf{M_2}$ were named as **C** and **D**.

*Partition and analysis of free and bound fatty acids*

The free and bound fatty acids partitioned from *Coffea benghalensis* B. Heyne Ex Schult were converted into their methyl ester derivatives and these were analyzed quantitatively by GLC[7,8].

*Evaluation of total antioxidant capacity*

A spectrophotometric method (phosphomolybdenum method) has been developed for the quantitative determination of antioxidant activity. In this process, reduction of Mo (VI) to Mo (V) occurred by the specimen analyte and a green phosphate/ Mo(V) complex formed subsequently at acidic pH. Here sample analyte were hexane, water, dichloromethane and methanol extracts. Lipid soluble and water-soluble antioxidant capacities were expressed as equivalents of ascorbic acid for samples of unknown composition[9].

*Evaluation of antimicrobial activity*

Antimicrobial activity of the different extracts of *Coffea benghalensis* B. Heyne Ex Schult leaves was studied against six pathogenic bacterial strains, three Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*) and three Gram-negative (*Escherichia coli*, *Salmonella typhi*, *Shigella flexneri)* bacteria whereas antimicrobial activity of the different extracts of *Coffea benghalensis* B. Heyne Ex Schult stems was studied against eight pathogenic bacterial strains, four Gram-positive and four Gram-negative bacteria and three Fungi. Hexane, dichloromethane, methanol, and water, extracts of *Coffea benghalensis* B. Heyne Ex Schult leaves and stems were used for antimicrobial activity test. As the reference, Ciprofloxacin (5µg/disc) standard disc was used. The antibacterial capacity of the test specimen was estimated by quantifying the diameter of zone of inhibition in term of ppm[10].

## III. Results and Discussion

*Quantitive estimation of caffeine*

The absorbance of the aqueous solution of the sample was measured at 276 nm against similar as taken for standard solutions. *Coffea benghalensis* B. Heyne Ex Schult seed is a source of caffeine which is a very important alkaloid for human being. It contains 34.22mg/100g dried powder seed. So caffeine can be extracted and used from the seed of *Coffea benghalensis* B. Heyne Ex Scult.

**Table 1. Determination of absorbance of caffeine against wavelength 276nm.**

| No. | Concentration of Ascorbic acid (mg/L) | Absorbance |
|---|---|---|
| 1 | Blank | 0.0 |
| 2 | 10 | 0.565 |
| 3 | 20 | 1.055 |
| 4 | 30 | 1.578 |
| 5 | 40 | 2.019 |
| 6 | 50 | 2.566 |
| 7 | *Coffea benghalensis* (Dichoromethane extract) | 0.921 |

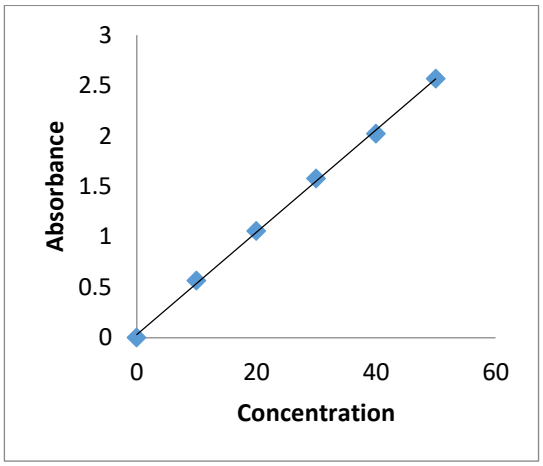


**Fig. 1.** A calibration curve for estimation of caffeine

*Phytochemical Screening*

Phytochemical screening was carried out on the leaves and stems of the plant during investigation and the presence of phytochemicals such as tannins, saponins, flavonoids, steroids, and cardiac glycosides were found.

*Characterization of Compound A*

The compound **A** was isolated as a white crystalline solid having $R_f$ value 0.60 (DCM: Hexane-80:20). It was soluble in dichloromethane and melting point was found 154-155$^0$C. After spraying with vanillin-sulfuric acid spray reagent, subsequently heating at 110$^0$C for several minutes, it appeared as purple color.

The **FT-IR** spectrum of **A** showed a broad absorption bands at 3435 cm$^{-1}$ (O-H group), 2917 and 2848 cm$^{-1}$ (aliphatic C-H stretching), 1637cm$^{-1}$ (>C=C<), 1464 and 1381 cm$^{-1}$ (-$CH_2$- and –$CH_3$), 1296 and 1060 cm$^{-1}$ (C-O stretching), l958 and 799 cm$^{-1}$(>C=C-H)[11, 12]. The **$^1$H-NMR** (400 MHz, in $CDCl_3$) spectrum of the isolated compound A showed peaks at δ 0.696 and 0.997 (s, methyl protons at H-18 and H-19),

3.512 (oxymethine proton, H-3α), 5.342 (m, olefinic proton (H-6) at C-6), δ 5.032 and 5.144 ppm (m, olefinic protons at H-22, H-23), 0.882, 0.832, and 0.793 ppm (3 doublets, methyl protons at C-20 and C-25), 1.242 ppm (s, $–CH_3$ protons at C-29),1.985 ppm (s, OH-3β at C-3), 1.015 – 2.276 ppm ($-CH_2-$ and >CH- protons). The **$^{13}$C-NMR** spectrum (100 MHz, in $CDCl_3$) of compound **A** revealed the presence of twenty nine (29) carbon signals. The signals at δ 140.75, 138.34, 129.29 and 121.77 (olefinic carbons), 42.35 and 36.53 ppm (two quaternary carbons), 21.24, 21.11, 19.42, 19.05 and 12.07 ppm (six methyl carbons) , 21.11, 24.32, 25.43, 28.94, 31.64, 31.95, 37.27, 39.79 and 42.28 ppm (nine methylene carbons), 31.95, 40.52, 50.16, 51.26, 55.98, 56.79 ppm (seven methine carbons), 71.88 ppm (oxymethine carbon)[11,12]. Equating the spectral data of compound **A** with the reported[13,14] value of $^1$H-NMR and $^{13}$C-NMR spectral data of stigmasterol, the structure of the compound was demonstrated as stigmasterol having the structure-

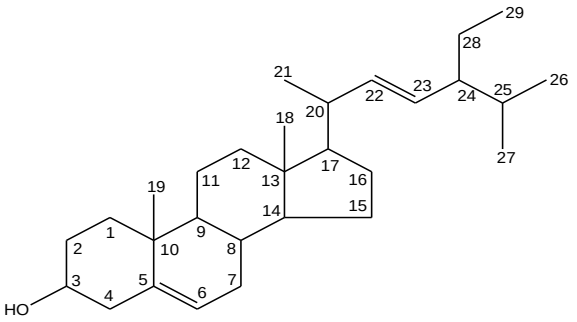


**Fig. 2.** Structure of compound A as stigmasterol

*Characterization of Compound B*

The compound **B** was isolated as a white crystalline solid having $R_f$ value 0.48 (DCM: Hexane-20:80) and its melting point was observed to be 42-43°C.

The **FT-IR** spectrum of **B** showed absorption bands at 3416 $cm^{-1}$ (O-H), 2918 and 2849 $cm^{-1}$ ($sp^3$ C-H, of either $–CH_3$, or $>CH_2$ group), 1607 $cm^{-1}$ (>C=C<.), 1472 $cm^{-1}$ C-H bend and 1098 $cm^{-1}$ (C-O stretching), 719 $cm^{-1}$ (long aliphatic chain),1261 $cm^{-1}$ ($-CH_3$)[11,12]. The **$^1$H-NMR** spectrum of compound **B** was recorded and its absorption frequencies were identified comparing the reported value of different stearates, oleates and glycerides. The $^1$H-NMR (400MHz, in $CDCl_3$) spectrum had δ 2.29 ppm (t, two protons of one $>CH_2$ at C-2 for H-2 proton), 1.60 ppm (methylene protons at C-3 for H- 3 proton), 1.24 ppm (proton at C-4 to C-17, H-4 to H-17), δ 0.86 ppm (t, methyl proton at C-18, H-18). The multiplate at 5.35 ppm indicated >C=C< double bonded two protons at C-9′ and C-10′ (H-9′ and H-10′). Due to the presence of proton at C-8′ and C-11′, the signal at δ 2.00 ppm was found. The doublet at δ 4.59 ppm was due to proton $–CH_2$-O- of glyceride at G-1 and G-3. The multiplate at δ 4.043(m) ppm indicated proton $–CH_2OH$ of glyceride at G-2. The **$^{13}$C-NMR** spectrum (100 MHz, in $CDCl_3$), of the compound **B** revealed the presence of 39 carbon signals. Due to carboxyl carbon (>C=O) of ester at C-1′, the signal at δ 174.06 ppm was observed. The signal at δ 172.34 ppm was due to carboxyl carbon (>C=O) of ester at C-1. Due to methylene ($-CH_2-$) carbon near to the carboxylate group at C-2′, the signal at δ 34.45ppm was found. The signal at δ 24.82 ppm was due to methylene ($-CH_2-$) carbon group at C-3′. The signal at δ 29.19 to 29.73 ppm indicates long chain ($-CH_2-$) which is marked at C-4′ to C-7′ and C-12′ to C-15′. The signal at δ 29.19 to 29.73 ppm indicated long chain ($-CH_2-$) which is marked at C-4 to C-15. The signal at δ 28.68 ppm was due to methylene ($-CH_2-$) carbon group at C-8′.The peak at δ 118.19 and 142.64 ppm was due to presence of olefinic (>C=C<) carbon marked at C-9′ and C-10′, respectively. The peak at δ 22.72 and 31.95 ppm was due to presence of methylene carbon marked at C-16′ and C-17′, respectively. The signal[11,12] at δ 14.14 ppm was indicative of terminal $–CH_3$ at C-18′. The compound **B** appeared as a glyceride of fatty acids from the physical characteristics and analyses of spectral (FT-IR, $^1$H-NMR and $^{13}$C-NMR) data of. Finally comparing the $^1$H-NMR and $^{13}$C-NMR data of compound B with those of reported[15,16] value the structure of the compound was established as 1, 3- steryl-oleyl-glyceride having the structure

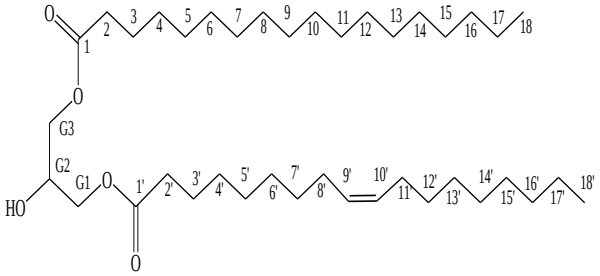


**Fig. 3.** Proposed structure compound B as 1, 3-stearyl-oleyl-glyceride

*Characterization of Compound C*

The compound **C** was a colorless liquid having $R_f$ value 0.70 (Hexane: DCM- 80:20).

The mass spectrum of the compound **C** showed molecular ion peak at m/z 270 which corresponds the molecular formula $C_{17}H_{34}O_2$. A peak was appeared at m/z 239 ([M-31]$^+$) due to loss of methoxyl group from the compound. This indicated that the compound was the methyl ester of a fatty acid. In the mass spectrum, a very intense peak at m/z 74 was observed due to McLafferty rearrangement. In addition to the McLafferty ion, there was a series of related ions formed by losses of neutral aliphatic radicals of genarel formula $[(CH_2)_nCOOCH_3]^+$ of which peak at m/z 87 is most abundant, followed by 101, 115, 129, 143 and so forth.A peak at m/z 255([M-15])$^+$) was observed due to loss of a methyl group from branch of long fatty acid chain. Comparing the mass spectral data of compound **C** with those of reported[17] value the structure of the compound was established as methyl 14-methylpentadecanoate having the structure-

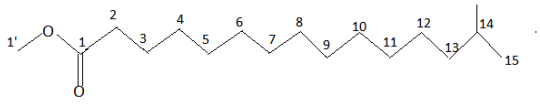

**Fig. 4.** Proposed structure compound C as Methyl 14-methyl pentadecanoate.

*Characterization of Compound D*

The compound **D** was isolated as a white crystalline solid having $R_f$ value 0.55 (DCM: MeOH-95:05). It was soluble in dichloromethane and melting point was found 270-274$^0$C. After spraying with vanillin-sulfuric acid spray reagent, subsequently heating at 110$^0$C for several minutes, it appeared as purple color.

The **FT-IR** spectrum of **D** showed a broad absorption bands at 3449 $cm^{-1}$ (O-H group), 2931 $cm^{-1}$ (aliphatic C-H stretching), 1633$cm^{-1}$ (>C=C<), 1467 $cm^{-1}$ (-$CH_2$- bending), 1252 $cm^{-1}$ (C-O stretching), 1023 $cm^{-1}$(>C=C-H)[11,12]. The **$^1$H-NMR** (400 MHz, in $CDCl_3$) spectrum of the isolated compound **D** showed peaks at δ 0.64 and 0.92 (s, methyl protons at H-18 and H-19), 3.50 (oxymethine proton, H-3α), 5.21 (m, olefinic proton (H-6) at C-6), δ 5.12 and 5.01 ppm (m, olefinic protons at H-22, H-23), 1.38, 0.85, and 0.80 ppm (three doublets, methyl protons at C-20 and C-25), 0.8 ppm (s, –$CH_3$ protons at C-29),1.985 ppm (s, OH-3β at C-3), 1.05 – 1.86 ppm (-$CH_2$- and >CH- protons), 4.23 ppm(d, H-1′) 3.01-3.67 ppm (m, H-2′ to H-6′ in glycoside unit) . The **$^{13}$C-NMR** spectrum (100 MHz, in $CDCl_3$+$CD_3OD$) of isolated compound **D** showed the presence of twenty nine (35) carbon signals. The signals at δ 140.06, 138.09, 129.04 and 121.86 (olefinic carbons), 43.01 and 36.47 ppm (two quaternary carbons), 18.99,18.62,19.43, 18.99, 11.72 and 11.86 ppm (six methyl carbons) ,40.25, 39.93, 37.01, 36.47, 32.05,31.64, 29.41, 28.39, 26.22, 24.07, and 20.72 ppm (eleven methylene carbons), 69.98, 56.61, 55.72, 51.03, 45.99, 36.39, 31.64 and 29.41 ppm (eight methine carbons), 69.98 ppm (oxymethine carbon), 100.8 ppm (anomeric C, C-1′), 69.98, 76.2, 73.31 and 76.22 ppm (four methine carbon, C-2′ to C-5′), 61.61 ppm (methelene C, C-6′)[11,12]. Comparing the spectral data of compound A with the reported value[18] of $^1$H-NMR and $^{13}$C-NMR spectral data of stigmasterol glucoside, the structure of the compound was established as stigmasterol glucoside having the structure-

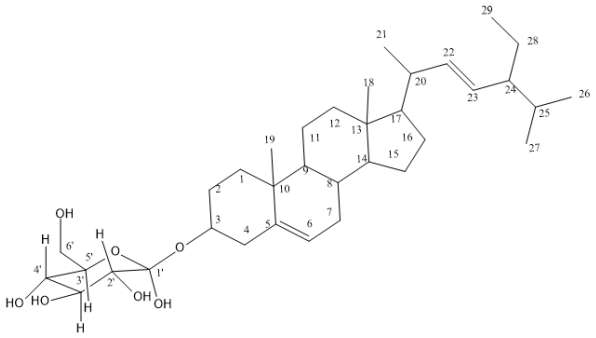

**Fig. 5.** Proposed structure compound D as stigmasterol glucoside.

Stigmasterol shows several pharmacological effect such as anticancer, anti-osteoarthritis, anti-inflammatory, antibacterial, anti-diabetic, immunomodulatory, antiparasitic, antifungal, antioxidant and neuroprotective properties[17]. Stigmasterol glucoside was found to be evincing high antioxidant activity and antifungal activity[19, 20]. Methyl 14-methyl *pentadecanoate was found to exhibit antibacterial* and *antifungal activity*[21].

*Fatty Acid Analysis*

From the fatty acid analysis, it showed that bound fatty acid is higher than that of free fatty acid (Table-1) which indicated the fatty acids are mainly present in the plant in the form of ester of glycerides. It appears from table 2, that the plant contains a mixture of saturated and unsaturated fatty acid. Palmiteolic acid was found to be higher in both BFA and FFA in leaves of *Coffea benghalensis* B. Heyne Ex Schult whereas palmitic acid was found to be higher in stems. The polyunsaturated fatty acids have been reported[22] to have a tendency to lower blood cholesterol level and are protective against cardiac arrhythmias. It also has beneficial effect on blood lipids, lowering pressure and serum cholesterol.

**Table 1. The sum of petroleum ether extract, bound and free fatty acids.**

| Species | Parts | Amount (g/100 g of dry powder) | | | |
|---|---|---|---|---|---|
| | | Petroleum ether extract (g) | Bound fatty acids (BFA) | Free fatty acids (FFA) | Total fatty acids |
| *Coffea Benghalensis* | Leaves | 0.575 | 0.072 | 0.003 | 0.075 |
| | Stems | 0.747 | 0.108 | 0.030 | 0.138 |

**Table 2. The total amount and relative percentages of bound and free fatty acids.**

| Fatty acid | Formula | *Coffea Benghalensis* leaves Relative percentage (%) | | *Coffea Benghalensis* stems Relative percentage (%) | |
|---|---|---|---|---|---|
| | | BFA | FFA | BFA | FFA |
| Capric | $C_{10}H_{20}O_2$ | 3.86 | 12.29 | - | - |
| Palmiteoleic | $C_{16}H_{30}O_2$ | 56.15 | 29.99 | - | - |
| Palmitic | $C_{16}H_{32}O_2$ | 8.79 | 2.98 | 70.55 | 52.18 |
| Stearic | $C_{18}H_{36}O_2$ | 8.83 | 12.37 | - | - |
| Oleic | $C_{18}H_{34}O_2$ | - | - | 19.91 | 23.38 |
| Linoleic | $C_{18}H_{32}O_2$ | 25.06 | 21.87 | - | - |
| Arachidic | $C_{20}H_{40}O_2$ | 1.83 | 12.18 | 6.27 | 15.88 |
| Behenic | $C_{22}H_{44}O_2$ | - | - | 3.26 | 8.56 |

*Evaluation of total antioxidant capacity*

Since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid, the phosphomolybdenum method is quantitative. Total antioxidant capacity of leaves of *Coffea benghalensis* B. Heyne Ex Schult was obtained for the methanol, water, ethyl acetate and n-hexane extracts as 287.6, 97.56, 22.76 and 20.56 ppm, respectively. Total antioxidant capacity of stems of *Coffea benghalensis* B. Heyne Ex Schult was obtained for the methanol, water, dichloromethane and n-hexane extracts as 95.04, 81.59, 66.03 and 60.26 ppm respectively. The synthetic antioxidant may contain impure chemicals. It has also carcinogenic effect. On the other hand, natural antioxidants are risk free and in purest form. So leaves of *Coffea benghalensis* B. Heyne Ex Schult can be used as a source of natural antioxidant[23,24].

*Evaluation of antimicrobial activity*

The hexane, water, dichloromethane and methanol extracts of *Coffea benghalensis* B. Heyne Ex Schult leaves exhibited moderate activity against the growth of the most of the test organisms (Table-3). The zones of inhibition produced by all these four extracts ranged from 9.5-16 mm, at a concentration of 800μg/disc.

The hexane, water, dichloromethane and methanol extracts of *Coffea benghalensis* B. Heyne Ex Schult stems exhibited poor activity against the growth of the most of the test organisms. The zones of inhibition produced by all these four extracts ranged from 7.0-8.0 mm, at a concentration of 400μg/disc. The experiment concludes that the hexane, dichloromethane, water and methanol extract of the species *Coffea benghalensis* B. Heyne Ex Schult leaves and stems have great potential as antimicrobial compounds against microorganisms[24].

**Table 3. Antimicrobial activity of the leaves extracts of *Coffea benghalensis* B. Heyne Ex Schult**

| Test bacteria | HE-1 (800 μg/disc) | DC-1 (800 μg/disc) | WA-1 (800 μg/disc) | ME-1 (800 μg/disc) | C.B.01 (800 μg/disc) | Ciprofloxacin (5μg/disc) |
|---|---|---|---|---|---|---|
| Zone diameter in mm for Gram positive Bacteria | | | | | | |
| *Bacillus cereus* | 15.5 | 10 | 12 | 16 | 10 | 32 |
| *Bacillus subtilis* | 13.5 | 8.5 | 12.5 | 15 | 9.5 | 30 |
| *Staphylococcus aureus* | 15 | 8 | 13 | 14.5 | 9 | 33 |
| Zone diameter in mm for Gram negative Bacteria | | | | | | |
| *Escherichia coli* | 14.5 | 9.5 | 12 | 15 | 10 | 35 |
| *Salmonella typhi* | 13 | 9 | 12.5 | 13 | 9 | 31 |
| *Shigellaflexneri* | 14.5 | 8.5 | 13.5 | 10 | 10.5 | 35 |

## IV. Conclusion

Phytochemical investigation of *Coffea benghalensis* B. Heyne Ex Schult leaves and stems revealed the presence of sterol such stigmasterol and stigmasterol glucoside and esters of fatty acids.34.22 mg caffeine content was estimated per 100 g seed powder. Unsaturated fatty acid palmiteoleic acid was found to be higher in leaves and saturated fatty acid palmitic was found to be higher in stems of *Coffea benghalensis* B. Heyne Ex Schult. Biological studies showed that, this plant exhibit moderate antioxidant and antimicrobial activity. So *Coffea benghalensis* B. Heyne Ex Schult can be a good source of medicinal interest for future.

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