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# CHARACTERIZATION OF BIOLOGICALLY ACTIVE FRACTIONS OF METHANOL EXTRACT OF MATURE LEAVES OF *POGOSTEMON BENGHALENSIS* (BURM. F.) KUNTZE AND *PERILLA FRUTESCENS* (L.) BRITTON

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**Keywords:**

Broad fraction, Activity index, Proportion index, Antioxidant, TLC

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**ABSTRACT:** The aim of the study was to determine the biologically active fractions of methanol extracts of mature leaves of *Pogostemon benghalensis* (burm. f.) Kuntze and *Perilla frutescens* L. Britton. The methanol extract was broadly fractionated sequentially in hexane, chloroform, acetone, ethyl acetate, and methanol. Activity index was recorded higher in methanol broad fractions of both of the plants. Broad fractions of both of the plants recorded a higher Proportion Index against *E. coli* (1.00). An increasing trend was recorded in the case of total phenol and flavonoid content with an increase in concentration from 1 mg/ml to 5 mg/ml. In case of *Pogostemon benghalensis* (Burm.f.) Kuntze, antioxidant inhibition of ascorbic acid against DPPH and ABTS are (98.28 ± 1.03% and 96.22 ± 2.01% respectively) which is higher than the crude extract (81.43 ± 0.09% and 72.32 ± 0.01% respectively) at 500 µl of the sample at 1mg/ml of concentration. TLC Spots from hexane and chloroform broad fraction recorded antibacterial inhibition against *B. subtilis*. $R_f$ values of TLC spots from hexane and chloroform broad fractions of both of the plants were recorded.

**INTRODUCTION:** The phytochemicals present in plants specify their special characteristic against various ailments. Preparation of herbal drugs is of great concern at present. Some specific compounds present in the plants indicate its special activity against some specific diseases. These compounds can be isolated and determined by various techniques. Isolation of these pure compounds needs an efficient method for separation from a mixture of compounds [1]. Chromatographic techniques and spectroscopic techniques are the most common and reliable techniques for the isolation of compounds from plants, which are skillfully exploited in the present study.

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The study was aimed to determine the biologically active fractions from methanol extracts of mature leaves of *Pogostemon benghalensis* (burm. f.) Kuntze and *Perilla frutescens* (L.) Britton. *Pogostemon benghalensis* (Burm. f.) Kuntze is commonly known as hookloti. Different parts of the plant have antibacterial, antifungal, antitubercular, antirheumatic properties [2, 3]. Various works have been carried out on this plant from different parts of the world [4-10].

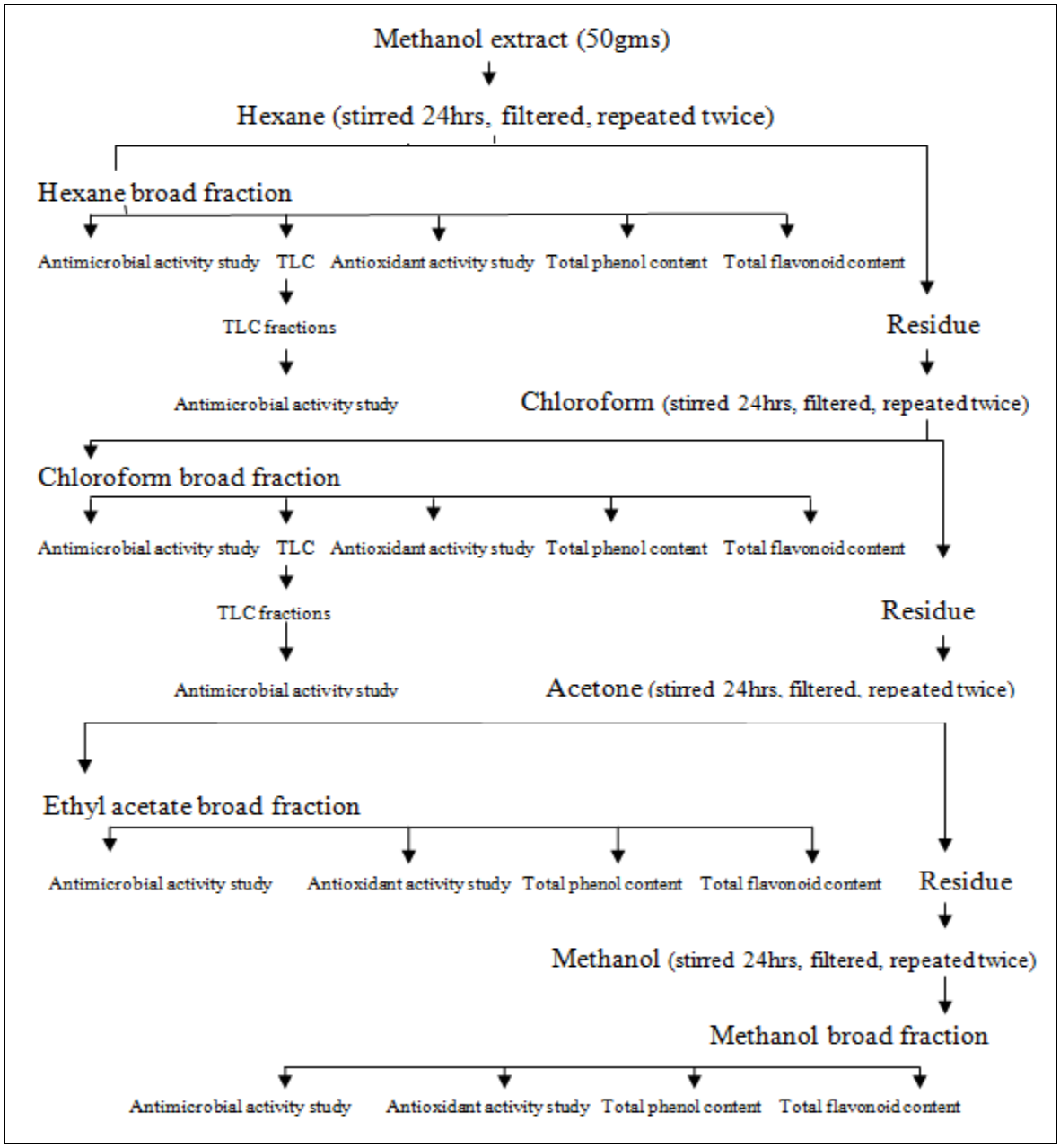
*Perilla frutescens* L. Britton is an evergreen aromatic shrub. It has aromatic leaves, shoots, and inflorescence, which are used for flavoring curries [11]. It is a traditional Chinese medicinal plant commonly used for a variety of diseases such as depression, inflammation, bacterial and fungal infections, allergy, intoxication, some intestinal disorders, and tumors [12, 13, 14]. Its health-promoting effects have been mainly attributed to its content of phenolic acids, flavonoids, and triterpenoids [15, 16].

It has antimicrobial, desensitizing, antiseptic, antipyretic, spasmolytic, antiseizure, antiasthmatic, antitussive, expectorant, restorative, tonic, anti-oxidant, anti-allergic, anti-inflammatory and anti-HIV-1 properties [17-27]. Various workers carried out experiments on this plant from various aspects [25, 28-35]. The plants are authenticated in the Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam and are authenticated as LSCDU5245 and LSCDU5246, respectively.

**MATERIALS AND METHODS:** Mature leaves of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton were collected from Dibrugarh University campus and extracted in methanol, which was further broad fractionated in hexane, chloroform, acetone, ethyl acetate, and methanol simultaneously.

The experiments were carried out in the year 2017-2018.

**Flowchart for Broad Fractionation:**




50 gm of methanol extract was taken in a conical flask, and broad fractionation was done using magnetic stir with the simultaneous use of solvents like- hexane, chloroform, acetone, ethyl acetate, and methanol. The broad fractions were kept separately for further work.

**Antimicrobial Activity Study of the Broad Fractions:** The broad fractions were dissolved in DMSO at a concentration of 5 mg/ml, and anti-microbial properties were tested out by agar well diffusion method, described by Nair *et al.,* [36] using 6 mm borer. The intensity of the activity was determined by measuring the diameter of the Zone of Inhibition (ZOI). Gram-positive and gram-negative bacterial strains are used in this experiment to know the antimicrobial activity.

**A. Gram-Positive Bacterial Strains:** *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermidis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744).

**B. Gram-Negative Bacterial Strains:** *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439).

**Determination of Activity Index (A.I.) of Broad Fractions:** Activity Index was determined by the method of Egharevba *et al.* [37] . It is the ratio of the mean of the diameter of ZOI obtained for the sample to the mean of the ZOI for the standard drug.

A.I. = Mean ZOI of the sample / Mean ZOI of the standard drug

An A.I. = 1 indicated that the plant sample was potent as the standard drug at the tested concentration.

**Determination of Proportion Index (P.I.) of Broad Fractions:** Proportion Index (P.I.) was determined by the method of Egharevba *et al.* [37]. It is the ratio of the number of positive results obtained for the sample to the total number of tests carried out.

Proportion Index= Number of a positive result obtained for the sample / Total number of tests carried out for each sample

**Determination of Total Phenol and Flavonoid Content of Broad Fractions:** Quantitative estimation of total phenol content and total flavonoid content of the five broad fractions were determined following the method of Malik and Singh, [38] and Mervat and Hanan, [39] respectively. Total phenol and total flavonoid content were determined in terms of mg Catechol Equivalent/gm of extract (mgCE/g extract) and mg Quercetin Equivalent/gm of extract (mg QE/g extract) respectively.

**Determination of Antioxidant Activity by DPPH and ABTS Radical Scavenging Activity:** DPPH and ABTS radical scavenging activity of the broad fractions were determined at a constant concentration of 500 µl by the method described by Anti-Stanojevic *et al.,* [40] and Re *et al*., [41] respectively.

**Separation of Broad Fraction by TLC:** The broad fractions are applied as spots in aluminum silica plate in a different solvent system. The solvent front was marked, and the $R_f$ value was determined. The chromatogram was observed in UV light (254 nm and 365 nm), and their color was developed using the iodine chamber.

**Antimicrobial Activity Study of TLC Spots from Hexane and Chloroform Broad Fraction:** Spots were scrapped off from the TLC plate and dissolved in hexane and chloroform, respectively. The solution was filtered, and dry mass of extract was recovered, which was dissolved in DMSO at a concentration of 5 mg/ml, and antimicrobial properties were tested out by agar well diffusion method, described by Nair *et al.,* [36] using 6 mm borer. The intensity of the activity was determined by measuring the diameter of the Zone of Inhibition (ZOI).

**Statistical Analysis:** One Way Analysis of Variance (ANOVA) was done with the percentage of antioxidant inhibition of the standard, crude and five broad fractions against DPPH and ABTS to know the significant difference between them.

## RESULTS AND DISCUSSION:

**Determination of Activity Index (A.I.) and Proportion Index (P.I.) of Five Broad Fractions of *Pogostemon benghalensis* (Burm. F.) Kuntze and *Perilla frutescens* (L.) Britton:** Activity Index (A.I.) and Proportion Index (P.I.) of the five broad fractions of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescence* L. Britton were calculated against Tobramycin (TOB) and Streptomycin (S), respectively, at 5mg/ml of concentration and are in the presented **Table 1-5**. In case of *P. benghalensis*, *B. cereus*, *P. vulgaris*, and *E. faecalis* did not show any activity, and their A.I. could not be determined. In the case of *S.*

*epidermidis*, a standard antibiotic did not show any activity. Methanol broad fraction have higher A.I. against *B. subtilis* (A.I. = 0.54), *S. aureus* (A.I. = 0.56) and *E. coli* (A.I. = 0.54) respectively in comparison with Tobramycin at 5 mg/ml concentration. All five broad fractions recorded A.I. against *B. subtilis* and *E. coli*. In comparison with Streptomycin (S), all five broad fractions recorded A.I. against *E. coli*. In the case of *P. frutescens* L. Britton acetone broad fraction did not record A.I. against *B. subtilis* in comparison with Tobramycin (TOB) and Streptomycin (S). Methanol (A.I. = 0.62) and ethyl acetate (A.I. = 0.54) broad fractions against *B. cereus* and methanol (A.I. = 0.56) broad fraction against *S. aureus* recorded higher A.I. in comparison to Tobramycin (TOB). Streptomycin (S) did not show any activity against *B. subtilis* and *S. epidermidis*; therefore A.I. cannot be determined in these cases. Methanol broad fraction against *S. aureus* (A.I. = 0.64), *P. vulgaris* (A.I. = 0.68), and *E. coli* (A.I. = 0.89) recorded higher A.I. than the other broad fractions.

The difference in antimicrobial activities recorded by the broad fractions are may be due to the compounds present in these fractions. The synergism between different compounds in the broad fractions may be the main reason for this difference in their activity. After fractionation, the compounds which are responsible for antimicrobial activity may be dissociated, which may cause less activity. The mixture of compounds in the crude extract may together cause higher antimicrobial activity than the broad fractions.

**TABLE 1: ZONE OF INHIBITION (ZOI) OF STANDARD ANTIBIOTIC TOBRAMYCIN (TOB) AND STREPTOMYCIN (S) AT 5 mg/mL OF CONCENTRATION**

| Test microorganisms | Tobramycin (TOB) (mm) | Streptomycin (S) (mm) |
|---|---|---|
| *B. subtilis* | 44±1 | - |
| *B. cereus* | 24±0 | 32±3 |
| *S. aureus* | 32±0 | 28±2 |
| *S. epidermis* | - | - |
| *E. coli* | 35±2.5 | 28±2 |
| *E. faecalis* | 42±2 | 60±2 |
| *P. vulgaris* | 40±1 | 22±2 |

Diameter of the cork borer = 6mm, 'x' indicates no inhibition

**TABLE 2: ACTIVITY INDEX (A.I.) OF FIVE BROAD FRACTIONS FROM *P. BENGHALENSIS* (BURM. F.) KUNTZE IN COMPARISON TO TOBRAMYCIN (AT 5 mg/mL CONCENTRATION)**

| Fractions | *B. subtilis* | A.I. | *B. cereus* | A.I. | *S. aureus* | A.I. | *S. epidermis* | A.I. | *P. vulgaris* | A.I. | *E. faecalis* | A.I. | *E. coli* | A.I. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methanol | 24±0 | 0.54 | - | NA | 18±2 | 0.56 | 15±0 | NA | - | NA | - | NA | 19±0 | 0.54 |
| Ethyl acetate | 11±1 | 0.25 | - | NA | 7±1 | 0.21 | 7±2 | NA | - | NA | - | NA | 12±2 | 0.34 |
| Acetone | 17±3 | 0.38 | - | NA | 9±0 | 0.28 | - | NA | - | NA | - | NA | 15±2 | 0.42 |
| Chloroform | 17±2 | 0.38 | - | NA | 9±0 | 0.28 | - | NA | - | NA | - | NA | 7±1 | 0.20 |
| Hexane | 15±2 | 0.34 | - | NA | - | - | 7±1 | NA | - | NA | - | NA | 7±1 | 0.20 |

Diameter of the cork borer=6mm, '-' indicates no inhibition

**TABLE 3: ACTIVITY INDEX (A.I.) OF FIVE BROAD FRACTIONS FROM *P. BENGHALENSIS* (BURM.F.) KUNTZE IN COMPARISON TO STREPTOMYCIN (AT 5 mg/mL CONCENTRATION)**

| Fractions | *B. subtilis* | A.I. | *B. cereus* | A.I. | *S. aureus* | A.I. | *S. epidermis* | A.I. | *P. vulgaris* | A.I. | *E. faecalis* | A.I. | *E. coli* | A.I. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methanol | 24±0 | - | - | NA | 18±2 | 0.64 | 15±0 | NA | - | NA | - | NA | 19±0 | 0.86 |
| Ethyl acetate | 11±1 | - | - | NA | 7±1 | 0.25 | 7±2 | NA | - | NA | - | NA | 12±2 | 0.54 |
| Acetone | 17±3 | - | - | NA | 9±0 | 0.32 | - | NA | - | NA | - | NA | 15±2 | 0.68 |
| Chloroform | 17±2 | - | - | NA | 9±0 | 0.32 | - | NA | - | NA | - | NA | 7±1 | 0.31 |
| Hexane | 15±2 | - | - | NA | - | - | 7±1 | NA | - | NA | - | NA | 7±1 | 0.31 |

Diameter of the cork borer=6mm, '-' indicates no inhibition

**TABLE 4: ACTIVITY INDEX (A.I.) OF FIVE BROAD FRACTIONS FROM *P. FRUTESCENS* (L.) BRITTON IN COMPARISON TO TOBRAMYCIN (AT 5 mg/mL CONCENTRATION)**

| Fractions | *B. subtilis* | A.I. | *B. cereus* | A.I. | *S. aureus* | A.I. | *S. epidermis* | A.I. | *P. vulgaris* | A.I. | *E. faecalis* | A.I. | *E. coli* | A.I. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methanol | 21±1 | 0.47 | 15±0 | 0.62 | 18±2 | 0.56 | 20±0 | - | 15±1 | 0.42 | 11±0 | 0.18 | 25±1 | 0.62 |
| Ethyl acetate | 15±1 | 0.34 | 13±3 | 0.54 | 10±0 | 0.31 | - | NA | - | NA | - | NA | 9±2 | 0.22 |
| Acetone | - | - | - | NA | 10±1 | 0.31 | - | NA | - | NA | 9±0 | 0.15 | 10.5±1.5 | 0.26 |
| Chloroform | 10±0 | 0.22 | - | NA | 8±1 | 0.25 | - | NA | - | NA | - | NA | 8±2 | 0.20 |
| Hexane | 11±2 | 0.25 | - | NA | - | NA | | NA | - | NA | - | NA | - | - |

NA- Not Applicable, '-' indicates no inhibition

**TABLE 5: ACTIVITY INDEX (A.I.) OF FIVE BROAD FRACTIONS FROM *P. FRUTESCENS* L. BRITTON IN COMPARISON TO STREPTOMYCIN (AT 5 mg/mL CONCENTRATION)**

| Fractions | *B. subtilis* | A.I. | *B. cereus* | A.I. | *S. aureus* | A.I. | *S. epidermis* | A.I. | *P. vulgaris* | A.I. | *E. faecalis* | A.I. | *E. coli* | A.I. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methanol | 21±1 | NA | 15±0 | 0.46 | 18±2 | 0.64 | 20±0 | NA | 15±1 | 0.68 | 11±0 | 0.18 | 25±1 | 0.89 |
| Ethyl acetate | 15±1 | NA | 13±3 | 0.40 | 10±0 | 0.35 | - | NA | - | NA | - | NA | 9±2 | 0.32 |
| Acetone | - | NA | - | NA | 10±1 | 0.35 | - | NA | - | NA | 9±0 | 0.15 | 10.5±1.5 | 0.37 |
| Chloroform | 10±0 | NA | - | NA | 8±1 | 0.28 | - | NA | - | NA | - | NA | 8± | 0.28 |
| Hexane | 11±2 | NA | - | NA | - | NA | | NA | - | NA | - | NA | - | NA |

NA- Not Applicable, '-' indicates no inhibition

Proportion Index (P.I.) of five broad fractions of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton are presented in Table 6-7. Ethyl acetate broad fraction against *B. subtilis* and *E. coli*, methanol broad fraction against *S. epidermidis* and *E. coli* and hexane broad fraction against *E. coli* of *P. benghalensis* recorded highest proportion Index (P.I. = 1.00). In case of *Perilla frutescens* methanol broad fraction against *B. subtilis* and *P. vulgaris*, ethyl acetate and acetone broad fraction against *E. coli* recorded highest (P.I.=1.00) Proportion Index.

**TABLE 6: PROPORTION INDEX (P.I.) OF FIVE BROAD FRACTIONS OF *P. BENGHALENSIS* (BURM. F.) KUNTZE**

| Fractions | *B. subtilis* | *B. cereus* | *S. aureus* | *S. epidermis* | *P. vulgaris* | *E. faecalis* | *E. coli* |
|---|---|---|---|---|---|---|---|
| Methanol | 0.75 | NA | 0.20 | 1.00 | NA | NA | 1.00 |
| Ethyl acetate | 1.00 | NA | 0.75 | 0.75 | NA | NA | 1.00 |
| Acetone | 0.50 | NA | 0.75 | NA | NA | NA | 0.50 |
| Chloroform | 0.50 | NA | 0.75 | NA | NA | NA | 0.50 |
| Hexane | NA | NA | NA | 0.50 | NA | NA | 1.00 |

NA- Not Applicable

**TABLE 7: PROPORTION INDEX (P.I.) OF FIVE BROAD FRACTIONS OF *P. FRUTESCENCE* L. BRITTON**

| Fractions | *B. subtilis* | *B. cereus* | *S. aureus* | *S. epidermis* | *P. vulgaris* | *E. faecalis* | *E. coli* |
|---|---|---|---|---|---|---|---|
| Methanol | 1.00 | 0.75 | 0.75 | 0.75 | 1.00 | 0.50 | 0.75 |
| Ethyl acetate | 0.75 | 0.75 | 0.50 | NA | NA | NA | 1.00 |
| Acetone | NA | NA | 0.50 | NA | NA | NA | 1.00 |
| Chloroform | 0.50 | NA | 0.50 | NA | NA | NA | 0.75 |
| Hexane | 0.75 | NA | NA | NA | NA | NA | NA |

NA- Not Applicable

**Quantitative Estimation for Total Phenol and Total Flavonoid Content (TPC and TFC) of Five Broad Fractions of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton:** The quantitative analysis was performed for total phenol and flavonoid content of five broad fractions of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton at different concentration (1mg/ml-5mg/ml) are presented in **Table 8-9**. In case of *Pogostemon benghalensis*, total phenol content of methanol broad fraction (5.01 ± 0.02 mgCE/g extract -24.12 ± 0.99 mgCE/g extract), ethyl acetate broad fraction (3.09 ± 0.00 mgCE/g extract- 7.00 ± 1.09 mgCE/g extract) and chloroform broad fraction (1.08 ± 0.30 mgCE/g extract- 2.89 ± 1.00 mgCE/g extract) recorded an increasing trend with the increase in concentration from 1 mg/ml to 5 mg/ml. Flavonoid content of acetone broad fraction (0.38 ± 0.01 mgQE/g extract- 2.00 ± 0.99 mgQE/g) also recorded an increasing trend with the increase in concentration from 1 mg/ml to 5 mg/ml. In *Perilla frutescens*, phenol content of methanol (12.01 ± 0.22 mgCE/g extract- 48.50 ± 0.01 mgCE/g extract), ethyl acetate (10.45 ± 0.02 mgCE/g extract- 19.21 ± 2.0 mgCE/g extract), acetone (4.05 ± 1.20 mgCE/g extract-6.90 ± 0.12 mgCE/g extract) broad fractions showed the increasing trend with the increase in concentration from 1 mg/ml to 5 mg/ml. Flavonoid content of methanol (2.20 ± 0.06 mgQE/g extract- 10.01 ± 1.09 mgQE/g extract), ethyl acetate (1.07 ± 0.03 mgQE/g extract- 2.78 ± 1.02 mgQE/g extract) and chloroform (1.09 ± 0.03 - 1.76 ± 0.01) broad fraction of *Perilla frutescence* also follow the increasing trend. Ojo *et al*., [42] also recorded the increasing trend in phenol content with the increase of concentration from 1 mg/ml to 5 mg/ml of ethanol extract of bark of *Blighia sapida*. They recorded phenolic content as 7.45 ± 0.09, 9.80 ± 0.13, 12.75 ± 0.11, 14.85 ± 0.01 and 19.92 ± 0.12

mg GAE/100gm at 1 mg/ml to 5 mg/ml of concentration respectively. This increasing trend may be due to proportionate increase of sample quantity thereby increasing the concentration of phytochemicals.

**TABLE 8: QUANTITATIVE ESTIMATION OF TOTAL PHENOL AND FLAVONOID CONTENT OF FIVE BROAD FRACTIONS OF *P. BENGHALENSIS* (BURM. F.) KUNTZE**

| Broad fractions | Total phenol content (mg catechol equivalent/gm dry extract) | Total flavonoid content (mg quercetine equivalent/gm dry extract) |
|---|---|---|
| **Methanol (concentration mg/ml)** | | |
| 1 | 5.01±0.02 | 1.10±0.05 |
| 2 | 9.21±1.20 | 3.01±0.13 |
| 3 | 13.49±0.43 | 4.14±1.30 |
| 4 | 21.00±2.01 | 3.98±0.01 |
| 5 | 24.12±0.99 | 6.00±0.00 |
| **Ethyl acetate (concentration mg/ml)** | | |
| 1 | 3.09±0.00 | 1.00±0.09 |
| 2 | 3.44±1.06 | 1.03±0.34 |
| 3 | 4.01±0.01 | 2.08±0.23 |
| 4 | 5.09±2.01 | 1.99±0.10 |
| 5 | 7.00±1.09 | 3.00±1.09 |
| **Acetone (concentration mg/ml)** | | |
| 1 | 2.03±0.09 | 0.38±0.01 |
| 2 | 4.40±1.04 | 1.08±0.20 |
| 3 | 3.01±1.00 | 1.11±0.14 |
| 4 | 3.99±0.01 | 1.98±0.01 |
| 5 | 4.90±0.08 | 2.00±0.99 |
| **Chloroform (concentration mg/ml)** | | |
| 1 | 1.08±0.30 | 0.67±0.19 |
| 2 | 1.20±0.00 | 1.09±0.22 |
| 3 | 2.01±0.08 | 0.67±0.09 |
| 4 | 2.71±0.01 | 2.09±0.01 |
| 5 | 2.89±1.00 | 2.98±0.08 |
| **Hexane (concentration mg/ml)** | | |
| 1 | 1.10±0.02 | 0.56±0.02 |
| 2 | 1.11±0.01 | 0.45±0.07 |
| 3 | 0.99±0.11 | 0.39±0.10 |
| 4 | 1.00±0.10 | 0.67±0.11 |
| 5 | 1.21±0.03 | 0.77±0.10 |

**Antioxidant Activity Study of Five Broad Fractions of *Pogostemon benghalensis* (Burm.f.) Kuntze and *Perilla frutescens* L. Britton against DPPH and ABTS:** Antioxidant activity of five broad fractions of *Pogostemon benghalensi* and *Perilla frutescens* against DPPH and ABTS at 500 µl of a sample at 1mg/ml of concentration are presented in **Table 10**. In case of *Pogostemon benghalensis*, antioxidant inhibition of ascorbic acid against DPPH and ABTS are (98.28 ± 1.03% and 96.22 ± 2.01%) which is higher than the crude extract (81.43 ± 0.09% and 72.32 ± 0.01%), methanol broad fraction (81.98 ± 1.67% and 80.33 ± 1.33%), ethyl acetate broad fraction (25.89 ± 1.89% and 49.01 ± 1.78%), acetone broad fraction (55.34 ± 2.00% and 45.20 ± 0.45%), chloroform broad fraction (38.56 ± 1.03% and 29.81 ± 0.34%) and hexane broad fraction (34.46 ± 0.56% and 42.02 ± 2.04%) respectively. In case of *Perilla frutescens*, ascorbic acid recorded higher antioxidant inhibition against DPPH and ABTS are (96.18 ± 1.03% and 89.00 ± 0.94%) than crude extract (75.23 ± 0.09% and 74.91 ± 2.45%), methanol broad fraction (79.34 ± 2.01% and 68.09 ± 3.04%), ethyl acetate broad fraction (28.67 ± 3.98% and 36.04 ± 0.05%), acetone broad fraction (56.90 ± 1.99% and 49.00 ± 1.09%), chloroform broad fraction (46.02 ± 2.00% and 46.24 ± 1.95%) and hexane broad fraction (40.98 ± 0.05% and 53.19 ± 1.00%).

**TABLE 9: QUANTITATIVE ESTIMATION OF TOTAL PHENOL AND FLAVONOID CONTENT OF FIVE BROAD FRACTIONS OF *P. FRUTESCENS* L. BRITTON**

| Broad fractions | Total phenol content (mg catechol equivalent/gm dry extract) | Total flavonoid content (mg quercetine equivalent/gm dry extract) |
|---|---|---|
| **Methanol (concentration mg/ml)** | | |
| 1 | 12.01±0.22 | 2.20±0.06 |
| 2 | 20.98±0.09 | 3.00±0.16 |
| 3 | 29.12±1.09 | 5.56±1.00 |
| 4 | 35.50±0.76 | 7.00±0.12 |
| 5 | 48.50±0.01 | 10.01±1.09 |
| **Ethyl acetate (concentration mg/ml)** | | |
| 1 | 10.45±0.02 | 1.07±0.03 |
| 2 | 12.09±1.09 | 2.11±0.22 |
| 3 | 12.98±0.04 | 2.34±1.01 |
| 4 | 14.09±1.09 | 2.78±0.99 |
| 5 | 19.21±2.00 | 2.78±1.02 |
| **Acetone (concentration mg/ml)** | | |
| 1 | 4.05±1.20 | 0.98±0.01 |
| 2 | 4.90±0.90 | 1.78±1.01 |
| 3 | 4.98±0.45 | 1.90±0.23 |
| 4 | 6.86±0.34 | 1.45±0.09 |
| 5 | 6.90±0.12 | 0.89±0.11 |
| **Chloroform (concentration mg/ml)** | | |
| 1 | 1.07±0.03 | 1.09±0.03 |
| 2 | 4.01±0.45 | 1.34±0.12 |
| 3 | 3.56±1.09 | 1.36±0.09 |
| 4 | 5.98±2.98 | 1.68±0.20 |
| 5 | 5.67±1.08 | 1.76±0.01 |
| **Hexane (concentration mg/ml)** | | |
| 1 | 0.45±0.01 | 0.35±0.02 |
| 2 | 1.98±1.08 | 0.37±0.19 |
| 3 | 1.56±0.03 | 0.19±0.21 |
| 4 | 2.09±0.30 | 0.67±0.06 |
| 5 | 2.10±1.04 | 0.56±0.03 |

One way Analysis of variance (ANOVA) showed that the inhibition percentage of the ascorbic acid,

crude extracts, and broad fractions are significantly different from each other at p<0.005 probability level. There is no significant difference between crude extract and methanol broad fraction of *P. benghalensis* against DPPH and ABTS; crude extract and methanol broad fraction against DPPH; chloroform and hexane broad fraction against DPPH; acetone and chloroform broad fractions against ABTS and acetone and hexane broad fractions against ABTS of *P. frutescens*.

**TABLE 10: ANTIOXIDANT ACTIVITY STUDY OF FIVE BROAD FRACTIONS OF *P. BENGHALENSIS* (BURM. F.) KUNTZE AND *P. FRUTESCENS* L. BRITTON AGAINST DPPH AND ABTS**

| Plants | | Percentage of inhibition at a concentration of 500µl of sample at 1mg/ml of conc. | |
|---|---|---|---|
| | | DPPH (%) | ABTS (%) |
| *P. benghalensis* | Ascorbic acid | 98.28±1.03*** | 96.22±2.01*** |
| | Crude extract | 81.43±0.09* | 72.32±0.01* |
| | Methanol broad fraction | 81.98±1.67* | 80.33±1.33* |
| | Ethyl acetate broad fraction | 25.89±1.89*** | 49.01±1.78*** |
| | Acetone broad fraction | 55.34±2.00*** | 45.20±0.45*** |
| | Chloroform broad fraction | 38.56±1.03*** | 29.81±0.34** |
| | Hexane broad fraction | 34.46±0.56*** | 42.02±2.04** |
| *P. frutescens* | Ascorbic acid | 96.18±1.03*** | 89.00±0.94*** |
| | Crude extract | 75.23±0.09* | 74.91±2.45*** |
| | Methanol broad fraction | 79.34±2.01* | 68.09±3.04*** |
| | Ethyl acetate broad fraction | 28.67±3.98*** | 36.04±0.05*** |
| | Acetone broad fraction | 56.90±1.99*** | 49.00±1.09*/** |
| | Chloroform broad fraction | 46.02±2.00** | 46.24±1.95** |
| | Hexane broad fraction | 40.98±0.05** | 53.19±1.00* |

**P<0.050, **=no significant *=no significant

**Separation of Broad Fraction by TLC:** For *Pogostemon benghalensis* (Burm. f.) Kuntze hexane broad fraction showed 13 spots in hexane: chloroform (3:2) solvent system and chloroform broad fraction recorded 10 spots in chloroform: acetone (1:9) solvent system, respectively. Similarly, in *P. frutescens* L. Britton hexane broad fraction recorded 14 bands using a solvent system, hexane: chloroform: acetone (1:3:2) and chloroform fraction showed 8 bands using a solvent system, chloroform: acetone (1:9) respectively. $R_f$ values TLC spots from hexane and chloroform broad fractions of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton are presented in **Table 11** to **14**.

**TABLE 11: $R_f$ VALUES OF TLC SPOTS FROM HEXANE BROAD FRACTION OF *P. BENGHALENSIS* (BURM. F.) KUNTZE**

| S. no. | Solvent distance (cm) | Spot distance (cm) | $R_f$ value |
|---|---|---|---|
| 1 | 6.2 | 0.4 | 0.0644 |
| 2 | 6.2 | 0.9 | 0.1451 |
| 3 | 6.2 | 1.3 | 0.2096 |
| 4 | 6.2 | 1.8 | 0.2903 |
| 5 | 6.2 | 2.0 | 0.3225 |
| 6 | 6.2 | 2.2 | 0.3548 |
| 7 | 6.2 | 2.8 | 0.4516 |
| 8 | 6.2 | 3.0 | 0.4838 |
| 9 | 6.2 | 3.4 | 0.5483 |
| 10 | 6.2 | 3.9 | 0.6290 |
| 11 | 6.2 | 4.3 | 0.6935 |
| 12 | 6.2 | 5.9 | 0.9516 |
| 13 | 6.2 | 6.1 | 0.9838 |

**TABLE 12: $R_f$ VALUES OF TLC SPOTS FROM HEXANE BROAD FRACTION OF *P. FRUTESCENS* L. BRITTON**

| S. no. | Solvent distance (cm) | Spot distance (cm) | $R_f$ value |
|---|---|---|---|
| 1 | 6.2 | 0.8 | 0.1290 |
| 2 | 6.2 | 1.4 | 0.2258 |
| 3 | 6.2 | 1.6 | 0.2580 |
| 4 | 6.2 | 2.3 | 0.3709 |
| 5 | 6.2 | 2.5 | 0.4193 |
| 6 | 6.2 | 2.8 | 0.4516 |
| 7 | 6.2 | 3.4 | 0.5322 |
| 8 | 6.2 | 3.8 | 0.6129 |
| 9 | 6.2 | 4.5 | 0.7096 |
| 10 | 6.2 | 4.9 | 0.7903 |
| 11 | 6.2 | 5.8 | 0.9354 |
| 12 | 6.2 | 5.9 | 0.9516 |
| 13 | 6.2 | 6 | 0.9677 |
| 14 | 6.2 | 6.15 | 0.9919 |

**TABLE 13: $R_f$ VALUES OF TLC SPOTS FROM CHLOROFORM BROAD FRACTION OF *P. BENGHALENSIS* (BURM. F.) KUNTZE**

| S. no. | Solvent distance (cm) | Spot distance (cm) | $R_f$ value |
|---|---|---|---|
| 1 | 18 | 0.8 | 0.0444 |
| 2 | 18 | 1.5 | 0.0833 |
| 3 | 18 | 2.3 | 0.1277 |
| 4 | 18 | 3.2 | 0.1777 |
| 5 | 18 | 4.6 | 0.2555 |
| 6 | 18 | 5.2 | 0.2888 |
| 7 | 18 | 6.0 | 0.3333 |
| 8 | 18 | 9.8 | 0.5444 |
| 9 | 18 | 12.5 | 0.6944 |
| 10 | 18 | 16.5 | 0.9166 |

Phytochemicals have different $R_f$ values in different solvent systems. The compounds having the same $R_f$ value was considered as the same compound.

**TABLE 14: $R_f$ VALUES OF TLC SPOTS FROM CHLOROFORM BROAD FRACTION OF *P. FRUTESCENS* L. BRITTON**

| S. no. | Solvent distance (cm) | Spot distance (cm) | $R_f$ value |
|---|---|---|---|
| 1 | 18 | 0.8 | 0.0444 |
| 2 | 18 | 1.4 | 0.0777 |
| 3 | 18 | 2.2 | 0.1222 |
| 4 | 18 | 4.5 | 0.2500 |
| 5 | 18 | 13.5 | 0.7500 |
| 6 | 18 | 15.4 | 0.8555 |
| 7 | 18 | 16.1 | 0.8944 |
| 8 | 18 | 16.6 | 0.9222 |

**Antimicrobial Activity Study of TLC Spots of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton:** Antimicrobial activity study of TLC spots of *Pogostemon benghalensis* (Burm. f.) Kuntze and *Perilla frutescens* L. Britton are presented in **Table 15** to **18**. Spots from hexane and chloroform broad fractions recorded antibacterial inhibition against *B. subtilis*. The spots did not record inhibition against other tested bacterial strains. On the other hand, crude extracts of these plants recorded antibacterial inhibition against these tested bacterial strains. The difference in antimicrobial activities recorded by the broad fractions and crude extracts are may be due to the compounds present in these fractions and extracts. The synergism between different compounds in the broad fractions may be the main reason for this difference in their activity.

**TABLE 15: ANTIMICROBIAL ACTIVITY STUDY OF TLC SPOTS FROM HEXANE BROAD FRACTION OF *P. BENGHALENSIS* (BURM. F.) KUNTZE**

| Sl. no. of spots | Zone of Inhibition (mm) | | |
|---|---|---|---|
| | *B. subtilis* | *S. epidermis* | *E. coli* |
| 1 | X | X | X |
| 2 | 12±1 | X | X |
| 3 | 12±0 | X | X |
| 4 | X | X | X |
| 5 | 10±1 | X | X |
| 6 | X | X | X |
| 7 | X | X | X |
| 8 | X | X | X |
| 9 | X | X | X |
| 10 | X | X | X |
| 11 | X | X | X |
| 12 | 11±1 | X | X |
| 13 | 10±1 | X | X |

Diameter of the cork borer=6mm, 'x' indicates no inhibition

After fractionation, the compounds responsible for antimicrobial activity may be dissociated, which may cause less activity. The mixture of compounds in the crude extract may together cause higher antimicrobial activity than the broad fractions.

**TABLE 16: ANTIMICROBIAL ACTIVITY STUDY OF TLC SPOTS FROM CHLOROFORM BROAD FRACTION OF *P. BENGHALENSIS* (BURM. F.) KUNTZE**

| Sl. no. of spots | Zone of Inhibition (mm) | | |
|---|---|---|---|
| | *B. subtilis* | *S. epidermis* | *E. coli* |
| 1 | 12±0 | X | X |
| 2 | 11±1 | X | X |
| 3 | 9±2 | X | X |
| 4 | X | X | X |
| 5 | X | X | X |
| 6 | X | X | X |
| 7 | 8±1 | X | X |
| 8 | 8±1 | X | X |
| 9 | 10±0 | X | X |
| 10 | X | X | X |

Diameter of the cork borer=6mm, 'x' indicates no inhibition

**TABLE 17: ANTIMICROBIAL ACTIVITY STUDY OF TLC SPOTS FROM HEXANE BROAD FRACTION OF *P. FRUTESCENS* L. BRITTON. AGAINST *B. SUBTILIS***

| Sl. no. of spots | Zone of Inhibition (mm) |
|---|---|
| | *B. subtilis* |
| 1 | X |
| 2 | X |
| 3 | X |
| 4 | 10±1 |
| 5 | 10±1 |
| 6 | X |
| 7 | 11±0 |
| 8 | 8±0 |
| 9 | X |
| 10 | X |
| 11 | X |
| 12 | X |
| 13 | 9±1 |
| 14 | 9±0 |

Diameter of the cork borer=6mm, 'x' indicates no inhibition

**TABLE 18: ANTIMICROBIAL ACTIVITY STUDY OF TLC SPOTS FROM CHLOROFORM BROAD FRACTION OF *P. FRUTESCENS* L. BRITTON**

| Sl. no. of spots | Bacterial strains Zone of Inhibition (mm) | | |
|---|---|---|---|
| | *B. subtilis* | *S. aureus* | *E. coli* |
| 1 | 8±0 | X | X |
| 2 | 9±0 | X | X |
| 3 | 8±0 | X | X |
| 4 | X | X | X |
| 5 | X | X | X |
| 6 | X | X | X |
| 7 | X | X | X |
| 8 | X | X | X |

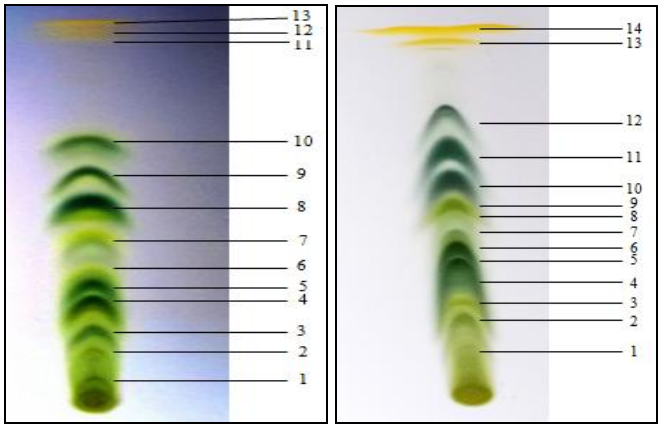



**PHOTO 1: TLC OF HEXANE BROAD FRACTIONS (60% HEXANE WITH CHLOROFORM) SHOWING SPOTS FROM *P. BENGHALENSIS* (BURM. F.) KUNTZE AND *P. FRUTESCENS* L. BRITTON**

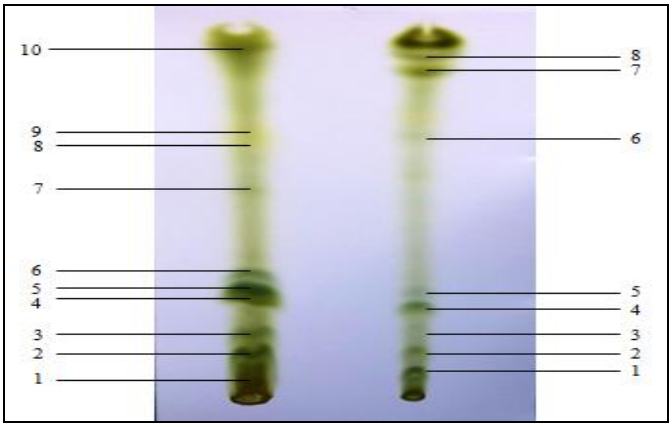



**PHOTO 2: TLC OF CHLOROFORM BROAD FRACTION (10% CHLOROFORM WITH ACETONE) SHOWING SPOTS OF *P. BENGHALENSIS* (BURM.F.) KUNTZE AND *P. FRUTESCENS* L. BRITTON**

**CONCLUSION:** From the above study, it can be concluded that the polar broad fractions from both of the plants recorded higher phenol and flavonoid content and antioxidant activity. Non-polar broad fractions established antibacterial activity against *B. subtilis.*

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