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Solvent-based comparative analysis of antioxidant and antimicrobial activities in *Calliandra haematocephala* extracts

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Abstract
Calliandra haematocephala flowers were evaluated for their phytochemical composition, antioxidant activity, and antimicrobial properties to explore their potential health benefits. The study employed both cold and hot extraction methods with various solvents to assess the presence of bioactive compounds. Phytochemical screening revealed the presence of flavonoids, tannins, and phenols. The antioxidant activity, evaluated through DPPH, FRAP, and ABTS assays, indicated that cold extracts generally exhibited superior antioxidant activity compared to hot extracts, with the highest activity observed in cold ethyl acetate and acetone extracts. Antimicrobial testing demonstrated notable activity of ethyl acetate extracts against Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. The results suggest that *Calliandra haematocephala* flowers possess significant antioxidant and antimicrobial properties, with cold extraction methods providing the most potent extracts. These findings underscore their potential for use in therapeutic applications and further research.

Keywords: Antimicrobial activity, antioxidant activity, extraction methods, phytochemical analysis, secondary metabolites, *Calliandra haematocephala*

1. Introduction

The aim of this study is to explore the phytochemical composition, antioxidant potential, and antibacterial activity of various extracts derived from *Calliandra haematocephala* flowers. *C. haematocephala*, originally native to North America, has been extensively cultivated in Indian gardens and parks for its diverse medicinal properties. Scientific investigations have revealed a multitude of beneficial effects associated with this plant, ranging from anti-inflammatory and gastroprotective properties to antitumor and anti-HIV activities [1]. Traditionally, it has been utilized for its antioxidant properties, as a blood purifier, and in the treatment of ailments such as hemorrhoids [2].

Pharmacological studies have highlighted the gastroprotective and immunomodulatory activities of *C. haematocephala* extracts, demonstrating promising results in mitigating gastric lesions and enhancing immune responses [3]. Furthermore, investigations into its cytotoxic effects against rotavirus infections have shown significant potential in reducing mortality and alleviating symptoms associated with viral infections [4]. Moreover, the plant exhibits notable antifungal and antimicrobial activities, indicating its potential as a natural therapeutic agent against various pathogens [2, 5].

The botanical description of *C. haematocephala* portrays it as a sprawling shrub with attractive red powder puff flowers, known for their ability to attract butterflies and hummingbirds. The flowers, typically visible from November to April, are clustered in spherical heads and possess vibrant pink to red stamens [1]. Despite the wealth of research on *C. haematocephala*, there remains a gap in understanding its phytochemical composition, antioxidant potential, and antibacterial activity, particularly focusing on its flower extracts. Therefore, this study aims to address these gaps by: Identifying and quantifying the phytochemical compounds present in various *C. haematocephala* flower extracts, providing insights into their chemical composition. Assessing the antioxidant activity of the flower extracts using multiple assays (DPPH, FRAP, ABTS), elucidating their potential in scavenging free radicals and combating oxidative stress-related diseases. Determining the total phenolic content of the flower extracts, which is indicative of their antioxidant capacity and health-promoting properties.

Quantifying the total flavonoid content of the flower extracts, shedding light on the abundance of flavonoids known for their diverse health benefits. Evaluating the antibacterial activity of the flower extracts against selected bacterial strains, potentially contributing to the development of natural antimicrobial agents.

2. Materials and Methods

Calliandra haematocephala flowers were meticulously collected from the University of Mumbai campus in the month of February. Following collection, the flowers underwent thorough washing under running tap water to remove dust and impurities and were then air-dried in shade until completely devoid of moisture. Subsequently, the dried flowers were finely powdered using a mechanical grinder for extract preparation.

Fig 1: *Calliandra haematocephala* flower

Two types of extracts were prepared: cold and hot extracts. For the cold extract, 10 g of dried powder was mixed with 20 ml of various solvents (petroleum ether, ethyl acetate, chloroform, acetone, methanol, ethanol, and distilled water), ground using mortar and pestle, and filtered using Whatman's filter paper No. 1, with the resulting filtrate stored in a clean bottle in the refrigerator. The hot extract was prepared through the Soxhlet apparatus extraction method, employing solvents like petroleum ether, ethyl acetate, chloroform, acetone, methanol, and ethanol. The extraction process spanned two days, with 200 ml solvent per round-bottom flask and 20 grams of powdered flower per thimble. Upon completion, the samples were concentrated to a volume of 40 ml and stored similarly. Additionally, a hot aqueous extract was produced by immersing 10g of dried powder in 20 ml distilled water, boiling it for 10–15 min, filtering, and refrigerating the filtrate for further analysis. These meticulously prepared samples are intended for subsequent scientific investigation and study.

2.1 Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the *C. haematocephala* flower powder under study were carried out in extracts using the standard procedures.

Test for Carbohydrates: Add 1 ml of extract and 1 ml of Benedict's reagent, then boil the mixture in a water bath for 5-10 minutes, resulting in green, yellow, or red coloration, indicating the presence of carbohydrates [6].

Test for Steroids: Combine 2 ml of extract with 2 ml of $CHCl_3$ and 2 ml of H_2SO_4, resulting in violet to blue-green coloration, indicating the presence of steroids [7].

Test for Protein: Mix 2 ml of extract with Ninhydrin reagent and heat in a boiling water bath for 10 minutes, resulting in a violet color ring or blue color, confirming the presence of protein [8].

Test for Amino Acids: Combine 2 ml of extract with 2 ml of Ninhydrin reagent and heat in a hot water bath for 20 minutes, resulting in the appearance of a purple color, indicating the presence of amino acids [8].

Test for Flavonoids: In 5 ml of diluted ammonia solution, add 2 ml of extract, and a few drops of concentrated H_2SO_4, resulting in yellow coloration disappearing on standing or blackish-red color, indicating the presence of flavonoids [9].

Test for Terpenoids: Combine 2 ml of extract with 2 ml of chloroform and 3 ml of concentrated H_2SO_4, resulting in a monolayer of reddish-brown color, confirming the presence of terpenoids [10].

Test for Cardiac Glycosides: Add 1 ml of extract, 1 ml of chloroform, and concentrated H_2SO_4, resulting in reddish-brown color, confirming the presence of cardiac glycosides [10].

Test for Tannins: Mix 2 ml of extract with 2 ml of water and add 2-3 drops of 5% $FeCl_3$, resulting in blue-black coloration, indicating the presence of tannins [6].

Test for Saponins: Combine 1 ml of extract with 1 ml of distilled water, then shake or vortex, resulting in froth appearing, confirming the presence of saponins [11].

Test for Phlobeatinins: Mix 2 ml of extract with 1% aqueous HCl, resulting in a red precipitate, confirming the presence of phlobeatinins [6].

Test for Fatty Acids: Combine 0.5 ml of extract with 5 ml of ether and allow it to dry on filter paper, resulting in appearance on transparency on filter paper, indicating the presence of fatty acids [6].

Test for Anthocyanins: Add 2 ml of extract with 2 ml of 2N HCl and ammonia, resulting in pink-red turning to blue-violet, confirming the presence of anthocyanins [7].

Test for Leucoanthocyanins: Mix 5 ml of extract with 5 ml of iso-amyl alcohol, resulting in an upper layer appearing red, indicating the presence of leucoanthocyanins [7].

Test for Coumarins: Combine 2 ml of extract with 3 ml of 10% NaOH, resulting in a green color, confirming the presence of coumarins [7].

Test for Phenols: Combine 2 ml of extract with 3 ml of ethanol and a pinch of $FeCl_3$, resulting in a greenish-yellow color, confirming the presence of phenols [7].

2.2 Total Phenolic Content

Total phenolic contents in the flower powder extracts were determined by Folin–Ciocalteu colorimetric method as described by scientists with some minor modifications [12]. Standard gallic acid solution was prepared by dissolving 1 mg of it in 10 ml of methanol (0.1 mg/ml). Various concentrations of gallic acid solutions in methanol were prepared from the standard solution. To each concentration, 5 ml of 10% Folin–Ciocalteu reagent (FCR) and 4 ml of 7% Na_2CO_3 were added making a final volume of 10 ml thus, the obtained blue colored mixture was shaken well and incubated for 30 min at 40 °C in a water bath. Then, the absorbance was measured at 760 nm against blank. The FCR reagent oxidizes phenols in flower extracts and changes into a dark blue color, which is then measured by UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Freshly prepared flower extract made various solvents. The procedure as described for standard gallic acid was followed, and absorbance for each extract was recorded. The samples were prepared in triplicate for each analysis, and the average

value of absorbance was used. Gallic acid is used as the reference standard compound and results are expressed as gallic acid equivalents (µg/ml) [13].

2.3 Total Flavonoids Content

The total flavonoid content of the flower extracts was determined using the aluminum chloride colorimetric method with certain modifications. A stock solution of quercetin (0.1 mg/ml) was prepared by dissolving 2 mg of quercetin in 20 ml of methanol. This stock solution was subsequently diluted to obtain various concentrations. The total flavonoid content was expressed as a quercetin equivalent using a linear equation derived from the calibration curve. Different solvents were employed to prepare the extracts. For the analysis, 0.6 ml of the diluted standard quercetin solutions or extracts was mixed separately with 0.6 ml of a 2% aluminum chloride solution. After thorough mixing, the resulting solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured at a wavelength of 420 nm using a UV-Vis spectrophotometer, with a blank used for reference. The standard protocol for quercetin analysis was applied to plant extracts, and the absorbance of each extract was measured and documented. The concentration of total flavonoid content in the test samples was determined using the calibration plot and expressed as milligrams of quercetin equivalent (QE) per gram of plant material. All determinations were performed in triplicate [14].

2.4 Antioxidant Activity

2.4.1. Dpph (2, 2-Diphenyl-1-Picrylhydrazyl)-Scavenging Activity

The measurement of the DPPH free radical scavenging activity was performed according to methodology described by scientists with slight modifications [15]. Methanol as a diluent. Freshly prepared, 2 mg Trolox was dissolved in 20 ml of methanol and working stock of 0.1 mg/ml was prepared as standard. DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in methanol; 0.5 mM solution of DPPH was prepared by dissolving 0.019 g in 100 ml of methanol. Freshly prepared, flower extracts of various solvents [16]. 1ml of 0.5mM DPPH solution in methanol was mixed with 1 ml of plant extract solution of varying concentrations from 10 to 100 µg/ml with an interval of 10 µg/ml. Corresponding Trolox was used as reference standard. Mixture of 1 ml methanol and 1ml DPPH solution used as a control and methanol is used as blank. The decrease in absorbance was measured at 517nm after 30 minutes inhibition in dark and using a UV-VIS spectrophotometer. The inhibition % was determined using the following formula.

Percentage inhibition = ((AB–AA)/ AB) ×100

Where, AB is absorbance of DPPH + methanol; AA is absorbance of DPPH + sample extract/Trolox.

2.4.2. Ferric Reducing Antioxidant Potential (FRAP) assay

The antioxidant capacity of the medicinal plants was estimated spectrophotometrically following the procedure described by scientists with some minor modifications [17]. FRAP reagent- Add 10 mM TPTZ in 40mM HCl, 20mM $FeCl_3$, 300 mM acetate buffer (pH 3.6) and mix in ratio of 1:1:10. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution and then warmed at 37 ºC before use. Freshly prepared, 10 mg Trolox dissolved in 20 ml of methanol and working stock of 0.5 mg/ml was prepared as standard. Different concentrations of standard (150 uL) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyl triazine complex] were then taken at 593 nm. The standard curve was linear between 100 to 500 µg/ml Trolox. 10 µl of flower extract was added in 2990 µl of FRAP reagent. The absorbance was then represented as Trolox equivalent mg per gram of sample [18].

2.4.3. ABTS *(*2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

The antioxidant capacity of *C. haematocephala* extracts was determined by ABTS radical cation decolorization assay. The ABTS cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 hours before use. ABTS solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. Trolox was used as standard substance. Stock solution (1mg/ml) of Trolox was prepared by dissolving 10 mg of Trolox in 10 ml of methanol. This stock solution was diluted serially to make various concentrations. 5 µl of volume from each concentration was added in 3.995 ml of diluted ABTS solution, the absorbance was measured at 30 min after the initial mixing. 5 µl of flower extract to 3.995 ml of diluted ABTS solution, the absorbance was measured at 734 nm after 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula,

ABTS scavenging effect (%) = ((AB–AA)/ AB) ×100

Where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/Trolox [19].

2.5 Antimicrobial Assay by Agar Well Diffusion Method

The Agar well diffusion method is widely used to evaluate the antimicrobial activity of flower extracts. The nutrient agar plate is bulk seeded by adding a volume of 200 µl the microbial inoculum in the agar. Three Gram negative organisms, *Escherichia coli, Pseudomonas aeruginosa* and *Serratia marcescens* and three Gram positive organisms, *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes* were used to check the antimicrobial activity. Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork borer, and a volume (200 µl) of the flower extract solution at 50% w/v is introduced into the well. The plates were kept at 4 ºC for diffusion of extract into the nutrient agar medium. Then, agar plates are incubated 37 ºC and the zone of inhibition was observed after 48 hours. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. All the determinations were carried out in triplicate [20].

3. Results

3.1. Phytochemical Analysis

The screening analysis was performed to identify various secondary metabolites which are present in *C. haematocephala* flower using petroleum ether, ethyl acetate, acetone, chloroform, ethanol, methanol, and distilled water as solvents. The phytochemical analysis of petroleum, ethyl acetate, acetone, chloroform, ethanol, methanol, and distilled water extracts revealed distinct compositions for both the hot and cold extracts. The hot petroleum extract contained carbohydrates, flavonoids, terpenoids, and coumarins, while the hot ethyl acetate extract exhibited carbohydrates, steroids,

flavonoids, terpenoids, cardiac glycosides, tannins, coumarins, and phenols. The hot acetone extract contained carbohydrates, amino acids, steroids, flavonoids, terpenoids, cardiac glycosides, tannins, and phenols. The hot chloroform extract exhibited carbohydrates, flavonoids, terpenoids, and coumarins. The hot ethanol extract displayed carbohydrates, amino acids, steroids, flavonoids, terpenoids, cardiac glycosides, protein, saponins, and phenols. The hot methanol extract showed carbohydrates, amino acids, steroids, flavonoids, cardiac glycosides, tannins, saponins, phlobatannins, and phenols. The hot distilled water extract contained carbohydrates, steroids, flavonoids, terpenoids, cardiac glycosides, tannins, saponins, and phenols. On the other hand, the cold petroleum extract contained carbohydrates, protein, and amino acids, while the cold ethyl acetate extract exhibited carbohydrates, steroids, amino acids, flavonoids, terpenoids, cardiac glycosides, and coumarins. The cold acetone extract contained carbohydrates, amino acids, steroids, flavonoids, terpenoids, cardiac glycosides, protein, saponins, fatty acids, and phenols. The cold chloroform extract exhibited the presence of amino acids. The cold ethanol extract showed carbohydrates, amino acids, steroids, flavonoids, terpenoids, cardiac glycosides, protein, fatty acids, coumarins, and phenols. The cold methanol extract contained carbohydrates, protein, amino acids, saponins, fatty acids, coumarins, and phenols. Lastly, the cold distilled water extract displayed carbohydrates, protein, amino acids, saponins, fatty acids, coumarins, and phenols.

3.2 Total Phenolic Content

The phenolic content assay was conducted on various cold and hot extracts prepared from *Calliandra haematocephala* flowers using different solvents.

The results showed a range of phenolic content levels in the extracts, with the highest content observed in cold aqueous extract (96.459 μg/ml). Following cold aqueous, the next highest phenolic content was found in the cold acetone extract (85.963 μg/ml), followed closely by the cold ethyl acetate extract (84.035 μg/ml) and cold ethanol extract (79.644 μg/ml). The cold methanol extract exhibited a moderate phenolic content level (73.753 μg/ml). On the other hand, the cold petroleum ether extract showed a relatively lower phenolic content (62.186 μg/ml). Finally, the cold chloroform extract exhibited the lowest phenolic content (11.098 μg/ml). Among the solvents tested, hot acetone exhibited the highest phenolic content, with a concentration of 97.316 μg/ml. Hot methanol and hot ethanol also demonstrated significant phenolic content, with concentrations of 97.638 μg/ml and 95.174 μg/ml, respectively. The hot ethyl acetate extract followed closely with a concentration of 94.210 μg/ml. Hot aqueous and HOT petroleum ether exhibited lower phenolic content compared to the a fore mentioned solvents, with concentrations of 95.067 μg/ml and 11.098 μg/ml, respectively. The hot chloroform extract showed the lowest phenolic content, with a concentration of 2.4227 μg/ml.

3.3 Total Flavaniod Content

The flavonoid content assay was conducted on various cold and hot extracts prepared from *Calliandra haematocephala* flowers using different solvents. The results of the assay revealed the presence of flavonoids in all the extracts, albeit with varying concentrations.

Among the solvents tested, cold petroleum ether exhibited the highest flavonoid content, with a concentration of 44.951 mg/g. Following cold petroleum ether, cold ethyl acetate was found to have the second-highest flavonoid content at a concentration of 19.961 mg/g. The extracts prepared using cold acetone, cold methanol, and cold ethanol showed similar flavonoid concentrations, with values of 17.065 mg/g, 18.486 mg/g, and 18.834 mg/g, respectively. Cold chloroform and cold aqueous extracts exhibited the lowest flavonoid content among the solvents tested, with concentrations of 9.5573 mg/g and 14.196 mg/g, respectively.

The order of flavonoid content, ranging from high to low, was as follows: petroleum ether > ethyl acetate > ethanol, methanol, acetone > distilled water > chloroform.

Among the tested solvents, the highest flavonoid content was observed in the hot ethyl acetate extract, with a concentration of 45.916 mg/g. Following closely behind, the hot acetone extract exhibited a similar high flavonoid content of 46.050 mg/g. The hot methanol extract displayed a moderate flavonoid content of 20.765 mg/g. Next, the hot ethanol extract showed a lower flavonoid content of 22.159 mg/g. The hot petroleum ether extract had a relatively lower flavonoid content of 11.729 mg/g. The hot chloroform extract exhibited a further decrease in flavonoid content, measuring 9.4502 mg/g. Lastly, the hot aqueous extract showed the lowest flavonoid content of 9.5307 mg/g.

3.4 Antioxidant Activity

3.4.1. Dpph (2, 2-Diphenyl-1-Picrylhydrazyl)-Scavenging Activity

The DPPH antioxidant assay was performed on extracts of *C. haematocephala* flower, comparing the cold and hot extraction methods. The antioxidant activity of the *C. haematocephala* flower extract, as measured by the ability to scavenge DPPH free radicals, was compared with the standard Trolox. The DPPH assay was conducted on various cold and hot extracts prepared from *C. haematocephala* flower powder using different solvents.

The cold petroleum ether extract exhibited a significantly higher antioxidant activity (94.28%) compared to the hot extract (20.24%). This suggests that the cold extraction method is more effective in extracting antioxidant compounds from *C. haematocephala* using petroleum ether as the solvent. Both the cold and hot ethyl acetate extracts displayed high antioxidant activities. However, the cold extract (95.63%) showed a slightly higher percentage than the hot extract (91.99%), indicating that the cold extraction method may be marginally more efficient in extracting antioxidants using ethyl acetate as the solvent. The cold acetone extract demonstrated a higher antioxidant activity (64.09%) compared to the hot extract (35.15%). This suggests that the cold extraction method is more suitable for extracting antioxidant compounds from *C. haematocephala* using acetone as the solvent. Interestingly, the hot chloroform extract (61.05%) exhibited significantly higher antioxidant activity compared to the cold extract (14.70%). Therefore, the hot extraction method seems to be more effective for extracting antioxidants from *C. haematocephala* flowers using chloroform as the solvent. The cold methanol extract demonstrated higher antioxidant activity (95.52%) compared to the hot extract (74.58%). This suggests that the cold extraction method is more suitable for extracting antioxidant compounds from *C. haematocephala* using methanol as the solvent. Both the cold and hot ethanol extracts displayed relatively high antioxidant activities. The cold extract (91.28%) showed a slightly higher percentage than the hot extract (79.74%), indicating that the cold extraction method may be more efficient in extracting antioxidants using ethanol as the solvent. The cold distilled water extract showed a higher

antioxidant activity (71.34%) compared to the hot extract (54.20%). Therefore, the cold extraction method appears to be more suitable for extracting antioxidant compounds from *C. haematocephala* using distilled water as the solvent.

3.4.2 Ferric Reducing Antioxidant Potential (Frap) Assay

The FRAP (Ferric Reducing Antioxidant Power) assay was performed on various cold and hot extracts obtained from *Calliandra haematocephala* flowers, using different solvents. The results of the assay revealed the antioxidant capacities of the extracts, expressed in micrograms per gram mg/g.

Among the solvents tested, the highest FRAP value was observed in the cold acetone extract, which exhibited a concentration of 461.834 mg/g. Following acetone, cold ethanol extract displayed the second highest FRAP value with a concentration of 366.177 mg/g. Cold ethyl acetate extract ranked third with a concentration of 414.307 mg/g, indicating a significant antioxidant potential. Cold methanol and cold petroleum ether extracts exhibited concentrations of 313.235 mg/g and 265.106 mg/g, respectively, which demonstrated moderate antioxidant activity. Cold aqueous extract and cold chloroform extract yielded the lowest FRAP values among the solvents tested. The cold aqueous extract had a concentration of 277.740 mg/g, while the chloroform extract showed a slightly lower concentration of 238.635 mg/g.

The highest FRAP value was observed for the extract prepared in hot acetone, which exhibited a concentration of 455.217 mg/g. The second highest FRAP value was obtained from the extract prepared in hot methanol, with a concentration of 437.168 mg/g. Following hot methanol, the extract prepared in hot aqueous exhibited a concentration of 420.924 mg/g, demonstrating considerable antioxidant activity. The hot ethyl acetate extract showed a FRAP value of 433.558 mg/g, positioning it in the middle range among the solvents tested. Next in line is the hot ethanol extract, with a concentration of 393.250 mg/g. The hot petroleum ether and hot chloroform extracts displayed the lowest FRAP values among the solvents tested, both with a concentration of 168.245 mg/g.

3.4.3 2, 2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid (Abts)

The ABTS assay was performed on cold and hot extracts prepared from *Calliandra haematocephala* flowers using various solvents. The extracts were evaluated based on their antioxidant activity, and the results are presented in descending order from highest to lowest percentage inhibition.

The highest antioxidant activity was observed in the cold acetone extract, which exhibited a percentage inhibition of 38.46%. Following closely, the cold ethyl acetate extract displayed a percentage inhibition of 38.15%. The cold petroleum ether extract showed a relatively lower but still significant percentage inhibition of 28.70%. Next, the cold chloroform extract demonstrated a percentage inhibition of 29.47%. The cold ethanol extract displayed a percentage inhibition of 11.7%. The cold methanol extract exhibited the lowest percentage inhibition at 3.10%. Lastly, the cold aqueous extract showed a percentage inhibition of 40.00%.

Among the solvents used, hot acetone exhibited the highest percentage inhibition of 41.75%. Following closely behind was the hot aqueous extract, which demonstrated a significant inhibitory effect of 46.11%. Moving on to the hot ethyl acetate extract, it showed a relatively high percentage inhibition of 38.15%. Similarly, the hot petroleum ether extract exhibited a substantial inhibitory effect of 30.93%. Next, we observed a moderate inhibition of free radicals in the hot ethanol extract, with a percentage inhibition of 27.7%. On the other hand, the hot chloroform and hot methanol extracts demonstrated lower inhibitory effects. The chloroform extract displayed a relatively low inhibition percentage of 4.21%, while the methanol extract exhibited the lowest inhibition of 2.32%.

3.5 Antimicrobial Assay by Agar Well Diffusion Method

Based on the present investigation, it was found that *Calliandra haematocephala* flowers contain an antimicrobial principle. The study evaluated the antimicrobial activity of different extracts obtained using various solvents and extraction methods. Among the extracts tested, the ethyl acetate cold and hot extracts exhibited the highest potency against several microorganisms.

The cold extract of ethyl acetate also demonstrated moderate activity against *P. aeruginosa*, *B. subtilis*, *S. pyogenes*, and *S. marcescens*, with zone diameters of 13, 13, 13, and 10 mm, respectively. No activity was observed against *E. coli*. Petroleum ether and distilled water cold extracts did not show any antimicrobial activity in this investigation.

The ethyl acetate hot extract showed the zones of inhibition against *B. subtilis*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *S. marcescens*, and *S. pyogenes*, with zone diameters of 21, 21, 20, 19, and 16 mm, respectively. However, no activity was observed against *S. aureus*. Petroleum ether and distilled water hot extracts did not show any antimicrobial activity in this investigation.

The cold extract of acetone exhibited a 10 mm zone of inhibition only against *B. subtilis*. Acetone hot extracts showed no activity against microorganisms. Chloroform hot and cold extracts showed activity against *B. subtilis*, with a zone diameter of 10 mm. No activity was recorded in methanolic cold extract.

The hot methanolic extract displayed activity against *E. coli*, *B. subtilis*, *S. aureus*, and *S. pyogenes*, with zone diameters of 12, 15, 13, and 13 mm, respectively.

The ethanolic cold extract showed activity against *B. subtilis*, with a zone diameter of 10 mm. The hot ethanolic extract demonstrated moderate potency against *E. coli*, *B. subtilis*, and *S. aureus*, with zone diameters of 10, 15, and 13 mm, respectively.

4. Discussion

This study aimed to evaluate the phytochemical composition, antioxidant activities, and antimicrobial properties of various extracts from *Calliandra haematocephala*. Different solvents (petroleum ether, ethyl acetate, acetone, chloroform, ethanol, methanol, and distilled water) were used for both hot and cold extraction methods. Phytochemical analysis revealed that the composition varied with the extraction method and solvent. Petroleum ether extracts contained carbohydrates, flavonoids, terpenoids, and coumarins in the hot extract, while the cold extract had carbohydrates, protein, and amino acids. Ethyl acetate extracts showed a wide range of compounds including steroids, flavonoids, terpenoids, cardiac glycosides, tannins, coumarins, and phenols. Acetone extracts were rich in similar compounds, with notable quantities of saponins and fatty acids in the cold extract. Chloroform extracts primarily contained amino acids in the cold extract, and carbohydrates, flavonoids, terpenoids, and coumarins in the hot extract. Ethanol and methanol extracts had a diverse range of phytochemicals, including saponins, phenols, and fatty acids. Distilled water extracts contained significant amounts of saponins, tannins, and phenols.

The antioxidant activities measured by DPPH, FRAP, and

ABTS assays indicated that cold extracts generally exhibited higher antioxidant activity than hot extracts, except for chloroform. In the DPPH assay, cold petroleum ether (94.28%), cold ethyl acetate (95.63%), and cold methanol (95.52%) showed the highest activities. The FRAP assay results were mixed, with cold acetone (461.834 µg/ml), hot methanol (437.168 µg/ml), and hot distilled water (420.924 µg/ml) showing high antioxidant capacities. The ABTS assay showed that hot distilled water (46.11%), hot acetone (41.75%), and hot ethyl acetate (38.15%) had high activities. Total phenolic content (TPC) and total flavonoid content (TFC) varied with the extraction method; cold extraction was more efficient for petroleum ether, ethyl acetate, chloroform, and distilled water, while hot extraction was better for acetone, methanol, and ethanol. TPC and TFC were highest in cold acetone (97.316 µg/ml) and hot acetone (46.050 µg/ml), respectively.

Antimicrobial assays demonstrated that ethyl acetate extracts exhibited the most significant antimicrobial activity, effective against multiple microorganisms. Conversely, petroleum ether and distilled water extracts showed no antimicrobial activity. Overall, the study suggests that the efficacy of the extraction method depends on the solvent used. Cold extraction generally results in higher antioxidant activity, except for chloroform. The findings highlight the potential of *C. haematocephala* as a source of bioactive compounds with significant antioxidant and antimicrobial properties. Further research, including toxicity studies and clinical trials, is needed to confirm these findings and explore their therapeutic potential.

The present findings agree with previous studies, such as those by Punnagai and Muthiah [1] regarding the antimicrobial activities of flavonoids and tannins, Moharram, Marzouk, Ibrahim, and Mabry [21] on antioxidant properties, and Li *et al.* [22] on the phenolic content of *C. haematocephala*. Kandylis [23] also noted the high ABTS activity of the plant, supporting the results of this study. The study's antimicrobial findings align with those of Abo-Elhamd *et al.* [24], indicating significant bioactive potential in different parts of the plant.

5. Conclusion

This study conducted a comprehensive phytochemical analysis and evaluated the antioxidant and antimicrobial activities of *Calliandra haematocephala* flower extracts using various solvents. The phytochemical screening revealed the presence of diverse secondary metabolites, including carbohydrates, flavonoids, terpenoids, steroids, phenols, and saponins, with varying compositions across hot and cold extracts. The total phenolic content was highest in the cold aqueous extract (96.459 µg/ml) and hot acetone extract (97.316 µg/ml), while the highest flavonoid content was observed in the cold petroleum ether (44.951 mg/g) and hot acetone (46.050 mg/g) extracts.

Antioxidant activity, assessed through DPPH, FRAP, and ABTS assays, demonstrated significant activity across different extracts, with the cold petroleum ether and cold ethyl acetate extracts showing notable DPPH radical scavenging activity (94.28% and 95.63%, respectively). The cold acetone extract exhibited the highest FRAP value (461.834 mg/g) and significant ABTS inhibition (38.46%).

The antimicrobial assay revealed that ethyl acetate extracts (both cold and hot) exhibited the highest antimicrobial potency against several microorganisms, with zone diameters up to 21 mm. Overall, *Calliandra haematocephala* flowers possess valuable phytochemicals with substantial antioxidant and antimicrobial properties, highlighting their potential for therapeutic applications.

6. Acknowledgement

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7. Conflict of Interest

The authors declare that they have no competing or conflict of interest.

8. References

1. Punnagai K, Muthiah NS. *In vitro* antibacterial activity of ethanolic extract of *Calliandra haematocephala* against selected bacterial strains. Biomedical and Pharmacology Journal. 2017;10(3):1279-1284.
2. Tiwari J, Shukla A. Investigations on *Calliandra haematocephala* flowers extract for in-vitro anthelmintic activity. Advance Pharmaceutical Journal. 2016;1(1):17-20.
3. Barbosa ADP. Gastroprotective and immunoadjuvant activities of butanolic extract of *Calliandra haematocephala*. Journal of Medicinal Plants Research. 2014;8:727-730.
4. Shaheen M, Mostafa S, El-Esnawy N. Anti-rotaviral effects of *Calliandra haematocephala* leaf extracts in-vitro and in-vivo. Journal of Virology and Antiviral Research. 2015;4(2):1-7.
5. Josephine I, Punnagai K. Antifungal activity of *Calliandra haematocephala* against selected pathogenic fungi: An in-vitro study. Journal of Clinical & Diagnostic Research, 2022, 16(1).
6. Raaman N. Phytochemical Techniques. New Delhi: New India Publishing Agency; c2006. p. 19-24.
7. Rao ND, Kaladhar DS. Biochemical and phytochemical analysis of the medicinal plant, *Kaempferia galanga* rhizome extracts. International Journal of Scientific Research. 2012;3:17-20.
8. Silva GO, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products. 2017;5(2):29-32.
9. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al.* Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 2008;7(3):1019-1024.
10. Sachin C, Arvind N, Vinesh D. The study of *in vitro* antimicrobial activity and phytochemical analysis of some medicinal plants in Chamoli Garhwal region. Pharmacognosy Journal. 2010;2(12):481-485.
11. Banso A, Adeyemo S. Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum*, *Bacopa monnifera*, and *Datura stramonium*. Biokemistri. 2016;18(1).
12. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Methods in Enzymology. Academic Press; c1999. p. 152-78.
13. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC.

The Scientific World Journal; c2020 .p. 1-2.
14. Chandra S, Khan S, Avula B, Lata H, Yang MH, ElSohly MA, *et al*. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evidence-Based Complementary and Alternative Medicine; c2014.
15. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995;28(1):25-30.
16. Nerdy N, Manurung K. Spectrophotometric method for antioxidant activity test and total phenolic determination of red dragon fruit leaves and white dragon fruit leaves. Rasayan Journal of Chemistry. 2018;11(3):1183-1192.
17. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry. 1996;239(1):70-76.
18. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 2006;19(6–7):669-675.
19. Rajurkar NS, Hande SM. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian Journal of Pharmaceutical Sciences. 2011;73(2):146-151.
20. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016;6(2):71-79.
21. Moharram FA, Marzouk MSA, Ibrahim MT, Mabry TJ. Antioxidant galloylated flavonol glycosides from *Calliandra haematocephala*. Natural Product Research. 2006;20(10):927-934.
22. Li AN, Li S, Li HB, Xu DP, Xu XR, Chen F. Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. Journal of Functional Foods. 2014;6:319-330.
23. Kandylis P. Phytochemicals and antioxidant properties of edible flowers. Applied Sciences. 2022;12(19):9937.
24. Abo-Elhamd AM, Aboul-Enein AM, Mohamed SM, Shalaby AS, Konsowa U, Hassan EM, *et al*. Chemical characterization, antioxidant and antihepatotoxic activities of *Calliandra haematocephala* (Hassk.), growing in Egypt. Journal of Chemical and Pharmaceutical Research. 2016;8(4):828-845.