Research in Plant Biology 2020, 10: 15-21
doi: 10.25081/ripb.2020.v10.6235
http://updatepublishing.com/journal/index.php/ripb

Research Article


ISSN: 2231-5101

# Characterization of nonpolar and polar solvent extracts from some tea plantation shade tree leaves with special reference to antioxidant and antibacterial activities

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## ABSTRACT

Beneficial properties of shade trees of tea plantations other than their medicinal properties have been extensively studied. This research was initiated to explore properties of some shade trees with special emphasis on their antioxidant and antibacterial properties. Leaves from shade tree like *Dalbergia sissoo* (DS), *Cassia siamea* (CS), *Derris robusta* (DR), *Leucaena leucocephala* (LL), *Acacia lenticularis* (AL) and *Melia azedarach* (MA) were used for the study. Characterization of shade tree leaves by determination of moisture, crude fibre and ash content and tests of non polar – polar solvent extracts for steroid, tannins, cardiac glycosides and coumarin, free radical scavenging, ferric reducing power, NO scavenging activities, quantification of Flavonoids and antibacterial activity were conducted. The average moisture, crude fibre and ash percentage of shade tree plants were found to be 62.95, 11.28 and 1.86 respectively. Methanol, ethanol, acetone and ethyl acetate respectively proved to be the most potent solvent for various phytochemical extractions as it gave positive results for tests like tannin, steroid, cardiac glycosides and coumarin. AL (91.46%), DR (92.69%), LL (94.32%) and MA (93.34%) leaf extracts showed high level of DPPH scavenging activity in their water extracts. In DS (88.11%) and CS (83.23%) maximum DPPH scavenging activity was observed in Diethyl ether and Methanol extracts respectively. Acetone extracts were more active than the water extracts in exhibiting ferric reducing power and NO scavenging activity. Summation of the quantity revealed that DS showed maximum presence of flavonoids and acetone as most potential for isolation of flavonoids. The decreasing order of summative antibacterial activity was recorded in DS, followed by CS, DR, AL, MA and LL. Chloroform showed the highest summative inhibition zone followed by ethanol, ethyl acetate, diethyl ether, acetone, water, hexane, benzene and methanol. Antioxidant and antibacterial potential of shade trees were established.

**Received:** May 10, 2020
**Accepted:** August 02, 2020
**Published:** August 30, 2020

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**KEYWORDS:** Tea plantation, shade tree, solvents, antioxidant, antibacterial

## INTRODUCTION

Tea, manufactured from the harvested fresh leaves of *Camellia sinensis* (L.) O. Kuntze, is the major plantation crop industry of India. Tea export earns a good amount of foreign exchange. In India tea plantations are large holding covering several hundred hectares of land. Presently, growing tea in small stretch of land has gained momentum, and these small tea growers contribute about half of the total produce. Tea is grown in hills as well in plains. In tea plantations located at lower altitudes and plains, shade trees are inseparable companion to the tea bushes and are considered an indispensable component for the thriving of the plantation. One or a few species of leguminous plants belonging to the genera *Acacia*, *Albizia*, *Dalbergia*, *Derris*, *Leucaena*, *Cassia* etc. are commonly planted as shade trees in the tea plantations of North East India. Presently, trees like *Melia azedarach*, *Cinnamomum tamala* etc. are also gaining popularity as candidates for fulfilling this role.

The part played by shade trees in the benefit of the plantation is multifaceted. They are primarily planted for the purpose of reducing evapotranspiration which helps with the regulation of temperature in tea gardens at lower altitudes and plains where temperatures rise beyond what is considered optimum.

In addition to this they serve to increase humidity at the bush level, add humus content to soil and improve nutrient status of the soil by the incorporation of nutrients from shed leaves by subsequently decomposition. They are utilized as well for pest and disease management. Thus, shade trees create a micro climate in the phyllospheric region of tea bushes that facilitates their proper growth and development. Besides influencing and improving tea plantation ecosystem, shade trees are also a potential source of firewood and timber in tea estates.

Previous studies showed that *Dalbergia sissoo* possesses antimicrobial, neural, cardiac, antioxidant, antiparasitic, antidiabetic [1], anti-inflammatory [1&2] and moderate analgesic activities [1&3]. *Melia azedarach* has antifungal and antibacterial properties [4]. *Cassia siamea* has been shown to cure liver problems, loss of appetite from gastrointestinal trouble, insomnia and is antihypertensive [5]. Central American people eat the young leaves, flowers, and young pods of *L leucocephala* in soups [6-8]. In addition, it is used as contraception, abortifacient to control stomach ache. The leaves have antihelmintic, antibacterial, antiproliferative and antidiabetic activities [8&9]. Even though these beneficial traits associated with shade trees have been studied, but there is no report on comparative medicinal activities of shade tree plants of tea plantations of North Bengal. Consequently, the present research work was designed to explore the comparative phytochemistry of some popularly employed tea plantation shade trees with special emphasis on their antioxidant and antibacterial properties.

## MATERIALS AND METHODS

### Collection of Plant Materials

Healthy, mature and disease free shade trees were selected from the tea plantation (Latitude 26.71109 and Longitude 88.35053) of University of North Bengal, Siliguri, West Bengal. Leaves from shade tree of *Dalbergia sissoo* Roxb. (DS), *Cassia siamea* Lam. (*Senna siamea* (Lam.)Irwin et Barneby) (CS), *Derris robusta* (Roxb. ex Dc. Benth. (DR), *Leucaena leucocephala* (Lam.)de Wit. (LL), *Acacia lenticularis* Benth. (AL) and *Melia azedarach* L. (MA) were collected early in the morning, packaged separately in ziplock bags and enclosed in an insulated box containing icepacks. Plant materials were identified from digital herbarium facility of Bioinformatics facility of the University.

### Determination of Moisture, Crude Fibre, and Ash Content

Moisture loss was determined by drying 10gm of shade tree leaves at 110°C for one hour. The difference in mass was expressed as percentage of moisture present in the samples. Presence of Crude fibres was conducted by acid and alkali digestion. 2 g of shade tree leaves ($W_0$) was boiled in 200 ml of concentrated sulfuric acid for 30 minutes. The acid extracts were filtered through muslin cloth and washed several times with distilled water until the pH of fibrous residue became neutral. Then the residue was boiled in 200ml of sodium hydroxide solution for 30 minutes and was filtered again through a muslin cloth. The extract was washed several times with distilled water until the pH of fibrous residue became neutral. The residue was then washed with 25ml of boiling 1.25% $H_2SO_4$ followed by washing thrice with 50ml distilled water and 25ml of ethyl alcohol. The residue was taken in a crucible and dried in a hot air oven for 2 hours at 130±2°C. The crucibles were brought down to room temperature [10]. The residue was cooled down and the masses recorded ($W_1$). Percentage of crude fibre content was calculated using the formula-

$$\text{Crude fibre (\%)} = (W_1/W_0)*100$$

Where weight of the leaves=$W_0$ and weight of the crude fibre = $W_1$.

For determination of ash content, 5gm of shade tree leaves were taken in crucibles and kept in muffle furnace at 600°C for 6 hours. At this temperature water evaporates from the sample and the rest of the contents burn down. Most of the minerals get convert to phosphates, sulphates and other oxides. Percentage of ash content was calculated by the formula-

$$\%\text{Ash} = (\text{weight of residue}/\text{weight of sample taken}) \times 100.$$

### Preparation of Extracts for Qualitative and Quantitative Analysis

The leaves were washed thoroughly in running tap water to remove adherent dust particles and finally washed with distilled water. Leaves were then dried with tissue paper and mechanically ground. 3gms of leaves from each sample was soaked with different non-polar to polar solvents (Hexane, Benzene, Chloroform, Di-ethyl ether, Ethyl acetate, Acetone, Ethanol, Methanol, and Water) for 48hours. The extracts were filtered through Whatman no.1 filter paper. The solvents were dried out at low temperature and finally dissolved in methanol at a concentration of 0.5gm/ml.

### Preliminary Phytochemical Group Screening of the Leaf Extracts

The preliminary phytochemical tests were carried out using standard phytochemical methods [11-14].For test of Steroid 0.5 ml of each leaf extract was evaporated and dissolved in 2ml chloroform. 2 ml of Conc.$H_2SO_4$ was introduced carefully by pouring down along the inner wall of the test tube. Formation of red color ring confirmed the presence of Steroid. For test of Tannins 0.5 ml of each leaf extract was taken in a clean glass test tube and a few drops of $HNO_3$ was added to it. The reddish to yellow color change of the solution indicated presence of Tannins. For test of Cardiac glycosides 0.5ml of each leaf extract was evaporated and dissolved in 1ml glacial acetic acid. 1drop of 10% $FeCl_3$ solution was added followed by 1ml of Conc. $H_2SO_4$ by pouring carefully down the side of test tube. Appearance of brown color ring at the interface indicated the presence of Cardiac glycosides. For test of coumarin1ml of each leaf extract was taken in test tube and a few drops of

NaOH solution was added to it. Yellow coloration indicated the presence of coumarin.

### Antioxidant Activity Assay

#### *2,2-diphenyl-1picrylhydrazyl(DPPH) free radical scavenging assay*

Free radical scavenging activity by DPPH assay was conducted following the protocol of Bhattacharya *et al*; 2009 [15]. 0.3ml of each shade tree leaf extract was added to 2 ml of methanol solution of 0.2mM DPPH. The mixture was vortexted vigorously and incubated at room temperature for 30minutes in the dark. Absorbance was measured at 517nm by UV-Vis spectrophotometer. Ascorbic acid (µg/mL methanol) was taken as a standard. DPPH scavenging percentage was calculated as-

Free radical scavenging %=[{(Control-Sample)/Control}×100].

#### *Ferric reducing power assay*

Ferric reducing power assay was done by following the protocol of Labar *et al*; 2019 [16]. To 200µl of each shade tree leaf extract, 500 µl phosphate buffer (pH 7.4) was added. Then, 1% 500 µl $K_2Fe(CN)_6$ was added and incubated for 20 minutes at 50°C. After that 10% 500µl Trichloroacetic acid, 1.5ml distilled water and 0.1% (W/V)300 µl ferric chloride were added. Then absorbance was measured at 700nm by UV-Vis spectrophotometer.

#### *Nitric Oxide (NO) scavenging activity*

To 1ml of each leaf extract, 10Mm 0.5ml sodium nitroprusside was added and kept at 25°c for 150 minutes. 1.2ml Griess reagent was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylene diamine was read at 546 nm by UV-Vis spectrophotometer [15].

#### *Quantification of flavonoids*

To 250µl of each leaf extract, 1.25ml double distilled water and 75µl of 5% $NaNO_2$ were added and kept at room temperature for 5minutes. Following this, 150µl of 10% $AlCl_3$ was added to it and kept for 6minutes at room temperature. After that 500µl of 1Mm NaOH and 275µl of double distilled water was added and the solution incubated for 30minutes. Finally absorbance was measured at 510nm by UV-Vis spectrophotometer [16,17].

### Antibacterial Activity

The leaf extracts in different solvents were dried and dissolved in 1ml of Dimethyl sulfoxide for antibacterial assay. Overnight grown cultures of *Staphylococcus aureus* (SA), *Bacillus subtilis* (BS), *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KN) were used for the present study. The antibacterial assay of the leaf extracts in different solvents was performed by well diffusion method [16]. Pour plates were made using the media (Mueller Hinton Agar) inoculated with 200µl inoculum. For the well diffusion method, 9 wells were made in the dish with a cork-borer and 100µl of each leaf extract in different solvents were pipetted into the wells. The Petridishes were then incubated overnight at 37°C. Antibacterial activity was discerned by measuring the diameter of the zone of inhibition surrounding the well containing the leaf extracts.

## RESULTS AND DISCUSSION

### Collection of Plant Materials

Environment plays a crucial role in plant metabolism, so molecules and their quantity vary with the habitat. Keeping this issue in mind, leaves of all the shade trees were collected from a small tea plantation within the campus of University of North Bengal. Leaves from shade trees of *Dalbergia sissoo* (DS), *Cassia siamia* (CS), *Derris robusta* (DR), *Leucaena leucocephala* (LL), *Acacia lenticularis* (AL) and *Melia azedarach* (MA) were collected in the morning hours to avoid accumulation of photosynthate and loss of water due to transpiration.

### Determination of Crude Fibre, Moisture and Ash Percentage

The average percentage of moisture, crude fibre and ash content of the six shade tree plants were found to be 62.95±3.1685, 11.28±0.0453 and 1.86±0.9835 respectively (Table:1). Except DR and CS all the other shade tree leaves contained moisture above the average. Maximum and minimum moisture percentages were recorded in MA (67.05%) and DR (57.70%) respectively. Except MA and CS all the other shade tree leaves contained crude fibre above the average.

Maximum and minimum crude fibre percentages were recorded in AL (16.65%) and MA (3.55%) respectively. Except DS, CS and MA all the other shade tree leaves contained ash content above the average. Ash content of DS was found to be 1% but comparatively higher percentage of it (19.3-26.1%) was reported in Iraq[1]. This could be due to different weather condition.

Maximum and minimum ash content was recorded in LL (3.15%) and MA (0.60%) respectively. Crude fibre of DS was found to be 12.5% but comparatively very higher percentage of it (8.43-12.0%) was reported in Iraq[1]. This could be due to less moisture in the environment and more crude fibre content.

Positive correlations were recorded only in between percentage of crude fibre and total ash content (0.6388), but moisture-crude

Table 1: Moisture, crude fibre and ash percentage of shade tree leaves

| Shade plants | Moisture % | Crude fibre % | Ash% |
|---|---|---|---|
| AL | 63.20±0.5458 | 16.65±0.4615 | 2.55±0.1357 |
| DR | 57.70±0.5029 | 14.40±0.3646 | 2.35±0.1601 |
| DS | 64.00±0.6107 | 12.50±0.1923 | 1.00±0.0984 |
| CS | 61.35±0.5114 | 9.55±0.3563 | 1.50±0.1581 |
| LL | 64.40±0.7395 | 11.05±0.3316 | 3.15±0.2474 |
| MA | 67.05±0.3258 | 3.55±0.1454 | 0.60±0.0651 |

fibre (-0.5986) and moisture-ash (-0.3864) content showed negative correlation.

## Preliminary Phytochemical Group Screening of the Leaf Extracts

Preliminary phytochemical screening of the leaf extracts for Steroid, Tannins, Cardiac glycosides and Coumarin are depicted in table 2. Methanol, ethanol, acetone and ethyl acetate respectively proved to be the most potent solvent for various phytochemical extractions as it gave positive results for tests like tannin, steroid, cardiac glycosides and coumarin. Preliminary phytochemical groups showed their varied presence in different shade tree species. Tannins in plant extracts can be pharmacologically useful as astringent [18]. They precipitates proteins in tissue leading to improvement of wound healing [19] and act as antibacterial [20] by inhibiting microbial proliferation by denaturation of enzymes involved in microbial metabolism. Tannin in much quantity was found in DR while DS shows good amount of cardiac glycosides. Cardiac glycosides were reported in LL [18]. Cardiac glycosides are used in the treatment of congestive heart failure and cardiac arrhythmia. Therefore all the plant extracts having cardiac glycosides could be used for this ailment. Steroid was found in good amount in DR, DS and CS. Presence of coumarin was maximum in DR followed by MA, LL, CS, DS and AL. In a previous investigation, LL was tested positive for tannin and reducing sugar [18].

Table 2: Preliminary phytochemical property of shade tree leaf extracts

| Solvent | Phytochemical groups | DR | LL | DS | AL | CS | MA |
|---|---|---|---|---|---|---|---|
| Hexane | Tannin | + | - | - | - | - | - |
| | Steroid | + | - | + | + | - | - |
| | Cardiac glycosides | + | + | - | + | + | - |
| | Coumarin | - | - | - | - | - | - |
| Benzene | Tannin | ++ | - | ++ | + | + | + |
| | Steroid | + | + | ++ | + | + | + |
| | Cardiac glycosides | + | + | + | + | + | + |
| | Coumarin | +++ | + | + | + | - | + |
| Chloroform | Tannin | ++ | + | + | ++ | + | + |
| | Steroid | + | + | ++ | ++ | ++ | + |
| | Cardiac glycosides | ++ | + | + | ++ | ++ | + |
| | Coumarin | +++ | +++ | - | - | +++ | +++ |
| Diethyl ether | Tannin | ++ | + | + | ++ | + | + |
| | Steroid | + | + | ++ | + | +++ | ++ |
| | Cardiac glycosides | ++ | + | ++ | + | ++ | + |
| | Coumarin | +++ | + | + | - | + | +++ |
| Ethyl acetate | Tannin | +++ | ++ | ++ | ++ | + | + |
| | Steroid | ++ | + | + | + | + | ++ |
| | Cardiac glycosides | ++ | ++ | ++ | + | ++ | + |
| | Coumarin | +++ | +++ | +++ | | +++ | +++ |
| Acetone | Tannin | +++ | ++ | ++ | ++ | ++ | + |
| | Steroid | +++ | ++ | ++ | + | ++ | +++ |
| | Cardiac glycosides | ++ | ++ | +++ | ++ | ++ | ++ |
| | Coumarin | +++ | +++ | +++ | +++ | +++ | +++ |
| Ethanol | Tannin | +++ | ++ | ++ | ++ | + | ++ |
| | Steroid | ++ | +++ | ++ | ++ | +++ | ++ |
| | Cardiac glycosides | ++ | ++ | +++ | + | ++ | ++ |
| | Coumarin | +++ | +++ | +++ | +++ | +++ | +++ |
| Methanol | Tannin | +++ | +++ | ++ | ++ | ++ | ++ |
| | Steroid | +++ | + | +++ | ++ | +++ | ++ |
| | Cardiac glycosides | ++ | ++ | +++ | + | ++ | + |
| | Coumarin | +++ | +++ | +++ | + | +++ | +++ |
| Water | Tannin | + | + | + | + | + | + |
| | Steroid | + | - | + | - | - | - |
| | Cardiac glycosides | + | + | + | + | + | + |
| | Coumarin | +++ | +++ | + | - | + | + |

## Antioxidant Activity Assay

### *2,2-diphenyl-1picrylhydrazyl(DPPH) free radical scavenging assay*

DPPH activity primarily indicates the ability of plant extracts to scavenge free radicals and this test is considered as an indicator of antioxidant activity of the extract. Non polar to polar solvent extracts of six shade tree leaf extracts showed variable potential of DPPH scavenging activity. Except the leaf extracts of DS and CS all other four shade trees, AL (91.46% or 96.882 µg AE / mL)), DR (92.69%), LL (94.32%) and MA (93.34%) leaf extracts showed high level of DPPH scavenging activity in their water extracts. In an similar research work by DPPH method, good result was recorded in methanol extracts of LL [21]. In DS (88.11%) and CS (83.23%) maximum DPPH scavenging activity was observed in Diethyl ether and Methanol extracts respectively. Lowest level of DPPH scavenging activities were observed in Hexane extracts of all the six shade tree leaves. DPPH scavenging activities were also low in Benzene except for the extracts of DS (55.49%) and DR (71.19%). Chloroform and Diethyl ether extracts of AL, CS and MA showed low activity but extracts of DS, DR and LL showed comparatively high scavenging activity. Ethyl acetate extract of AL (22.88%) showed low DPPH scavenging activity but in DS (61.24%), DR (65.49%), LL (87.33%), CS (48.22%) and MA (52.96%) the activities of ethyl acetate extracts were moderate or high. Acetone, Ethanol, Methanol and Water extracts of all the shade tree leaves showed moderate, high or very high DPPH scavenging activities. Results depicted in table 3 shows that solvent preference for antioxidant molecules vary from plant to plant. They have a general preference for polar solvents. Water may be considered as ideal solvent for extraction of free radical scavenging molecules from leaves of AL, DR LL and MA, while for DS and CS the ideal solvents are Diethyl ether and Methanol respectively.

### *Ferric reducing power and NO scavenging activity assay*

DPPH scavenging activity alone cannot be considered as the sole test to assess antioxidant activity of plant extracts. So, Ferric reducing power and NO scavenging activity assays were also conducted. Ferric reducing power and NO scavenging activity assay were conducted for Acetone and water extracts only as they responded well in DPPH scavenging activity. The results of Ferric reducing power and NO scavenging activity assay are represented in table 4. In all the shade tree leaves, the acetone extracts were more active than the water extracts in exhibiting ferric reducing power and NO scavenging activity. In acetone extracts of shade tree leaves the FRPA potential increased with increase in concentration for DS, LL, CS and MA while a decrease was observed in case of

Table 3: DPPH scavenging percentage and equivalent Ascorbic acid scavenging activity of shade tree leaf extracts

| Solvents | AL | | DS | | DR | | LL | | CS | | MA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *DPPH | **AE | DPPH | AE | DPPH | AE | DPPH | AE | DPPH | AE | DPPH | AE |
| Hexane | 5.19± 0.1069 | <1 | 8.36± 0.1011 | 2.019 | 42.57± 0.4072 | 41.07 | 10.75± 0.5052 | 4.747 | 7.59± 0.1844 | 1.140 | 4.85± 0.3772 | 1 |
| Benzene | 12.32± 0.5536 | 6.539 | 55.49± 0.9372 | 55.820 | 71.19± 0.5169 | 73.743 | 14.64± 0.2762 | 9.188 | 8.8± 0.1527 | 2.521 | 11.21± 0.1637 | 5.272 |
| Chloroform | 28.64± 0.3252 | 25.170 | 64.46± 0.4563 | 66.060 | 74.89± 0.4021 | 74.966 | 91.54± 0.4508 | 96.973 | 25.84± 0.3655 | 21.973 | 10.9± 0.3914 | 4.918 |
| Diethyl ether | 42.51± 0.4257 | 41.003 | 88.11± 0.6058 | 93.058 | 66.83± 0.4250 | 68.765 | 89.21± 0.3593 | 94.313 | 13.92± 0.1950 | 8.366 | 25.52± 0.2369 | 21.608 |
| Ethyl acetate | 22.88± 0.3027 | 18.594 | 61.24± 0.2523 | 62.384 | 65.49± 0.5109 | 67.236 | 87.33± 0.8311 | 92.167 | 48.22± 0.4968 | 47.521 | 52.96± 0.3555 | 52.932 |
| Acetone | 86.7± 0.4119 | 91.448 | 55.12± 0.6023 | 55.398 | 68.62± 0.4618 | 70.809 | 80.63± 0.3153 | 84.519 | 76.2± 0.3910 | 79.462 | 80.31± 0.4554 | 84.154 |
| Ethanol | 74.01± 0.9003 | 76.962 | 75.32± 0.4063 | 78.457 | 57.58± 0.5056 | 58.206 | 81.01± 0.2909 | 84.953 | 72.19± 0.2400 | 74.884 | 76.93± 0.1715 | 80.295 |
| Methanol | 90.98± 0.3300 | 96.334 | 81.34± 0.4576 | 85.329 | 74.85±0 .5488 | 77.921 | 89.36± 0.1400 | 82.769 | 83.23± 0.6404 | 87.487 | 82.39± 0.2474 | 86.528 |
| Water | 91.46± 0.5225 | 96.882 | 86.32± 0.2969 | 91.014 | 92.69± 0.2793 | 98.286 | 94.32± 0.2650 | 100.147 | 54.97± 0.3050 | 55.227 | 93.34± 0.2797 | 99.028 |

*DPPH (% scavenging) and **Ascorbic acid equivalent (µg AE / ml) was calculated from the standard curve, y=0.876x+6.591, $R^2$ = 0.993

Table 4: Ferric reduction power assay (FRPA) and NO scavenging activity of solvent extracts

| Assay (0.4mg/µl) | Solvent | Sample (µl) | AL | DS | DR | LL | CS | MA |
|---|---|---|---|---|---|---|---|---|
| FRPA | Acetone | 100 | 3.6918±0.0394 | 3.6708±0.0139 | 4.2777±0.0272 | 4.4078±0.0862 | 3.7656±0.1608 | 3.5960±0.1447 |
| | | 200 | 3.5716±0.0248 | 4.2783±0.0279 | 4.1864±0.1037 | 5.3983±0.2489 | 3.8924±0.0674 | 3.6356±0.0226 |
| | Water | 100 | 3.4003±0.0286 | 3.4449±0.0734 | 4.1684±0.1053 | 3.8934±0.0421 | 3.2953±0.0253 | 3.4839±0.0166 |
| | | 200 | 3.5040±0.0448 | 3.2412±0.0995 | 3.3765±0.1013 | 3.7269±0.0734 | 3.4641±0.0114 | 3.5965±0.0227 |
| NO | Acetone | 100 | 2.7239±0.0160 | 3.6175±0.0663 | 2.5277±0.0448 | 2.4831±0.0422 | 2.5391±0.1210 | 2.5058±0.1131 |
| | | 200 | 3.2012±0.1164 | 3.7775±0.0321 | 3.1062±0.0575 | 3.0242±0.5299 | 2.9645±0.3542 | 2.9129±0.0509 |
| | Water | 100 | 1.8264±0.1331 | 1.8115±0.1096 | 2.0017±0.0653 | 1.9244±0.0319 | 1.9685±0.1016 | 1.8982±0.0465 |
| | | 200 | 1.8031±0.1187 | 1.8850±0.1112 | 1.9133±0.0507 | 1.8987±0.1118 | 1.8663±0.1680 | 1.8957±0.1651 |

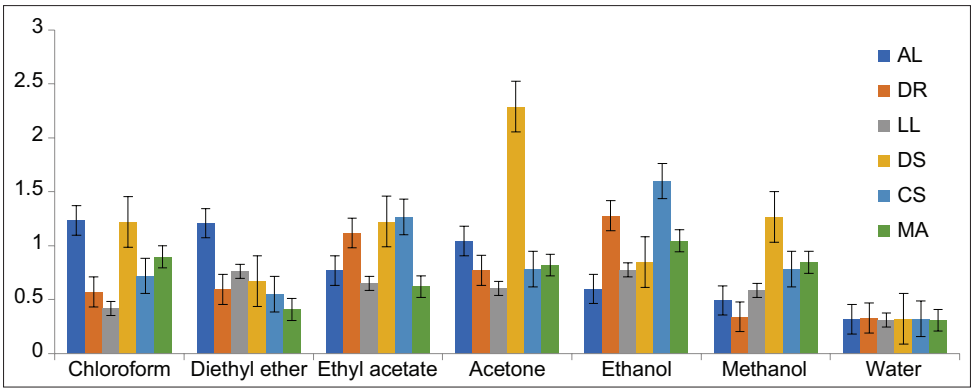


**Figure 1:** Quantity of flavonoids as quercetin equivalent detected in solvent extracts

AL and DR. In water extracts of shade tree leaves the FRPA potential increased with increase in concentration for AL, CS and MA but decreased in DS, DR and LL. Among the shade tree leaves experimented on, maximum FRPA was observed in acetone extracts of LL at concentration of (0.4mg/µl) while the lowest activity was noted in water extract of DS at the same concentration. It was observed that the shade tree leaf extracts with acetone showed better NO scavenging activity than water extracts. In acetone extracts of all the shade tree leaves the NO scavenging activity increased with increase in concentration, but in water extracts of these shade tree leaves the NO scavenging activity increased with increase in concentration only in DS and decreased in AL, DR, LL, CS and MA. Among the shade tree leaves experimented on, maximum NO scavenging activity was observed in acetone extracts of DS at concentration of (0.4mg/µl) while the lowest activity was recorded in water extract of AL at the same concentration.

### *Quantification of flavonoids*

Flavonoids are considered as important antioxidant molecules that appear to possess a variety of mechanisms of action including free radical scavenging [22]. The presences of flavonoids in the extracts are tested for their antioxidant activity. Flavonoids are anti-allergic, anti-inflammatory, antimicrobial and anti-cancer [23]. Quantification of Flavonoids was conducted in Chloroform, Diethyl ether, Ethyl acetate, Acetone, Ethanol, Methanol and Water extracts of shade tree plant leaves. Determination of total flavonoid content expressed as mg Quercetin equivalent (QE)/g in fresh leaves of shade tree extracted by different solvents (chloroform-water). The quantities of flavonoids detected were converted to quercetin equivalent and are represented in figure 1. The shade tree and the solvents responded with much variability. Maximum amount of flavonoids were recorded in acetone extracts of DS (2.288 QE)/g) while lowest in water extract of MA (0.307QE)/g). Summation of the quantity of flavonoids in all the solvents revealed that DS (1.119 QE)/g) showed maximum while LL (0.585 QE)/g) showed minimum quantity of flavonoids. Similarly, summation of the quantity of flavonoids in all the shade trees revealed, Acetone (1.05 QE)/g) showed maximum while water (0.317 QE)/g) showed minimum quantity of flavonoids.

| Solvent | Organism | AL | DR | DS | CS | LL | MA |
|---|---|---|---|---|---|---|---|
| Hexane | EC | 0.3 | 0.3 | 0 | 0.6 | 0 | 0 |
| | KP | 0.45 | 0.2 | 0.35 | 0.2 | 0.1 | 0 |
| | BS | 0 | 0 | 0.05 | 0 | 0.15 | 0 |
| | SA | 0.3 | 0.17 | 0.3 | 0 | 0 | 0.2 |
| Benzene | EC | 0.4 | 0 | 0.3 | 0 | 0 | 0 |
| | KP | 0.45 | 0.4 | 0.5 | 0 | 0.2 | 0.1 |
| | BS | 0.1 | 0 | 0 | 0 | 0.1 | 0.45 |
| | SA | 0 | 0.2 | 0.35 | 0 | 0 | 0 |
| Chloroform | EC | 0.3 | 0 | 0.45 | 0 | 0 | 0.3 |
| | KP | 0.5 | 0 | 0.5 | 0 | 0 | 0.3 |
| | BS | 0 | 1.6 | 0 | 1.5 | 0 | 0 |
| | SA | 0 | 0.17 | 1.35 | 0 | 0 | 0.2 |
| Diethyl ether | EC | 0.4 | 0 | 0.4 | 0.45 | 0 | 0.4 |
| | KP | 0 | 0.3 | 0.5 | 0 | 0 | 0.1 |
| | BS | 0 | 0.5 | 0.2 | 0 | 0.2 | 0.3 |
| | SA | 0 | 0.2 | 0.3 | 0 | 0 | 0.1 |
| Ethyl acetate | EC | 0.6 | 0.25 | 0.6 | 0 | 0 | 0 |
| | KP | 0 | 0 | 0.4 | 0 | 0 | 0.3 |
| | BS | 0 | 0.6 | 0 | 1.3 | 0 | 0.2 |
| | SA | 0.3 | 0.25 | 0.6 | 0 | 0.4 | 0 |
| Acetone | EC | 0.4 | 0.2 | 0.8 | 0 | 0 | 0.3 |
| | KP | 0.4 | 0 | 0.1 | 0 | 0 | 0 |
| | BS | 0 | 0 | 0 | 0.75 | 0 | 0.35 |
| | SA | 0.2 | 0.2 | 0.55 | 0 | 0 | 0 |
| Ethanol | EC | 0.4 | 0.5 | 0.9 | 0 | 0 | 0.3 |
| | KP | 0 | 0.35 | 0.4 | 0 | 0 | 0.5 |
| | BS | 0 | 0 | 0 | 1.8 | 0 | 0.3 |
| | SA | 0 | 0.17 | 0.4 | 0.2 | 0.3 | 0 |
| Methanol | EC | 0.3 | 0 | 0.5 | 0 | 0 | 0.35 |
| | KP | 0 | 0 | 0.7 | 0.3 | 0 | 0 |
| | BS | 0 | 0 | 0 | 0 | 0 | 0.2 |
| | SA | 0 | 0.25 | 0.35 | 0.35 | 0.1 | 0.1 |
| Water | EC | 0.3 | 0.3 | 0.2 | 0.4 | 0 | 0 |
| | KP | 0.3 | 0 | 0.5 | 0 | 0 | 0.55 |
| | BS | 0.35 | 0 | 0 | 0 | 0 | 0 |
| | SA | 0 | 0.5 | 0.2 | 0 | 0 | 0.2 |

**Figure 2:** Heatmap representing inhibition zones produced by non polar-polar solvent extracts of shade tree leaves

### Antibacterial Activity

The extracts obtained from the shade tree leaves using nine non polar to polar solvents exhibited varied degree of inhibition zones against two gram positive and two gram negative bacteria. The result of bacterial growth inhibition experiment has been represented by heatmap (Figure 2). The

summation of zones of inhibition produced by the extracts against the organisms showed that there is no strict preference against gram positive and negative bacteria. The inhibition zone produced by EC (12.2cm), BS (11cm), KP (9.95cm) and SA (9.475cm) were close in magnitude. Maximum summative antibacterial activity was recorded in DS (25.35cm) followed by CS (16.7cm), DR (15.775cm), AL (12.1cm), MA (12.0cm) and LL (2.95cm) with the least. The non-polar to polar solvents responded unequally towards extraction of antibacterial molecules from the shade tree leaves and the results were exhibited in the inhibition zones they produced against bacterial growth. Chloroform (7.175cm) showed the highest summative inhibition zone followed by ethanol (6.525cm), ethyl acetate (5.8cm), diethyl ether (4.35cm), acetone (4.25cm), water (3.8cm), hexane (3.675cm), benzene (3.55cm) and methanol (3.5cm).

## CONCLUSION

Tea being the most important plantation based industry of India forms the backbone of economy in some regions of the country. Trees planted to provide shade to tea bushes may have potential medicinal activities that may be exploited. Shade trees are rich in steroid, tannins, cardiac glycolsides and coumarin. High level of antioxidants and antibacterial activities were detected in some of the shade trees. Shade trees have preferences for solvents as all the solvent extracts were not found to equally potential for all the tests. Solvents like water (AL, DR LL and MA), diethyl ether (DS) and methanol (CS) for extraction of free radical scavenging molecules, acetone for flavonoids and chloroform for anti bacterial molecules were found to be useful. Thus we can clearly infer that selection of ideal solvent is essential for isolation or *in vitro* studies. So, the collective information of this research work could promote future research to utilize shade trees of tea plantations in an efficient manner. This initiative may further glorify tea industry by promoting more employment and income opportunities.

## ACKNOWLEDGEMENT

The authors would like to acknowledge the scientific team of Digital Herbarium Facility of Bioinformatics Facility, University of North Bengal for identifying the plant specimens.

## AUTHOR CONTRIBUTIONS

AG, SM and SS carried out the collection, experiments and data analysis. AG wrote the first draft of the manuscript. MB conceived the idea of the study, participated in its design and coordinated to draft the manuscript. All authors read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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