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Research Article

EFFECT OF SOLVENT WITH VARYING POLARITIES ON PHYTOCHEMICAL EXTRACTION FROM MATURE TEA LEAVES AND ITS EVALUATION USING BIOCHEMICAL, ANTIMICROBIAL AND *IN-SILICO* APPROACHES

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ABSTRACT

Research in tea has been limited to processed or packaged tea. Owing to its economical as well as medicinal importance, our work is focused on phytochemical extraction from mature fresh tea leaves based on extracting solvents with varying polarities with the prime focus on bio activity study using biochemical, microbial and *in-silico* approaches. Potent solvents were screened from different qualitative, quantitative and antioxidant tests. Antimicrobial screening was done along with its validation using GC-MS and *in-silico* approaches. Acetone extracts was found to be the most potent solvent for extraction followed by methanol and ethanol. Acetone and methanol extracts showed antagonist activity against *Staphylococcus* sp. with Minimum Inhibitory Concentration of 4 mg/ml and 8 mg/ml respectively. Gas chromatography - mass spectrometry identified bioactive compounds like Phenol, 3, 5-bis (1, 1-dimethylethyl), caffeine and Vitamin E as the probable compounds as antibacterial agents. Further *in-silico* results validated phenol as the most potent antimicrobial compound with its binding affinity of -7.2 kcal/mol to *S. aureus* DNA gyrase. Elaborate qualitative and quantitative phytochemical profiling thus gave an idea about the potency of particular solvent in extracting specific group of compounds. Bioactivity of potent extract against *Staphylococcus* sp. was recorded out of other bacterial strains investigated. GC-MS analysis of extracts gave insight into the type of compounds extracted. Further *in- silico* results provided interesting insights into the ability of phenol to bind against *S. aureus* DNA gyrase.

Keywords: Polarities, Phytochemicals, Phenol, docking, gyrase

INTRODUCTION

Tea the most popular health beverage enriched with important phytochemicals belongs to the genus *Camellia* under Theaceae family. The important phytochemicals in tea includes the polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline etc.), volatile oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), inorganic elements (e.g., aluminum, fluorine and manganese etc.) with polyphenols being the most important compound of pharmacological importance[1]. It has already been proved in series of experiments that abundant polyphenols in tea imparts many health protecting activities[2]. These compounds have a wide range of pharmaceutical properties which includes anti oxidative, anti carcinogenic and anti arteriosclerotic property[3-6]. Polyphenolic compounds present in tea may reduce the risk of a variety of illnesses, including cancer, coronary heart disease, atherosclerosis, high blood cholesterol concentrations and high blood pressure. Most of the research work has been focused on made tea or processed tea putting a limitation as such to tea plant[7].

The objective of this work mainly focuses on the qualitative and quantitative phytochemical screening as well as studying the antioxidant and antimicrobial activity of fresh mature leaves of five different clones of *Camellia sinensis* based on extracting solvents of different polarities ranging from non polar to polar.

Very little work has been done on phytochemical screening of fresh leaves of tea using range of organic solvents with research being limited to standard solvents and processed tea. Extraction method should ensure complete extraction of the desired compounds of interest without any chemical modification[8]. Extraction and determination of biologically active compounds depends upon the type of solvent used where solvents will diffuse into solid plant tissue and solubilize compound with same polarity[9]. Aqueous mixtures of ethanol, methanol and acetone, water, are commonly exploited to extract plants[10]. Researchers have reported use of aqueous methanol, acetone and ethanol[6,11], absolute methanol[12], absolute ethanol[13] and boiling water for the extraction of polyphenols from green, black and mate teas[14]. Different solvents like water, aqueous ethanol in different extracting time has been employed to extract phenolics from green and white tea[15].

MATERIAL AND METHODS

Plant selection

Five tea elite clones were selected as experimental material, namely TS569 (S1), China (S2), AV2 (S3), P312 (S4) and Assam (S5), the details of which are provided in Supplementary Table 1. The samples were collected from Mirik hills of Darjeeling district and was identified by Taxonomy lab, of Botany Department, University of North Bengal (Accession no- 10339).

Solvent selection

Nine different solvents in increasing order of polarity namely hexane, benzene, chloroform, diethylether, ethyl acetate, acetone, ethanol, methanol and cold water were chosen as extracting solvents.

Sample extraction

Fresh leaves of the samples were washed thoroughly under running tap water, air-dried and then pulverized using a grinder. The sample was weighed and 3 g each was distributed equally and immersed in 30 ml each of nine different solvents ranging from non polar to polar. After 48 hours the aqueous cold extracts was centrifuged and the supernatant thus collected was dried and stored in 4°C for future use.

Qualitative screening of phytochemicals

Qualitative test for phytochemicals included test for flavonoid, tannin, steroid, terpenoid, cardiac glycoside, diterpenes, coumarin, reducing sugar, protein, and saponin with slight modification[16-18]. The method is given in Supplementary Table 2.

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

As mentioned previously[19], DPPH was used to determine the antioxidant activity of the mixture of compounds extracted employing different solvents. The decrease in absorbance is marked by the free radical scavenging property of the compound, which donates hydrogen atom and scavenges the unpaired electron of the stable free radical of DPPH. To 100 µl of plant extracts (5 mg/ml) prepared from different solvents, 1900 µl of methanol was added and shaken. The mixture was incubated at room temperature for 30 minutes in dark. The absorbance was then recorded at 520 nm using spectrophotometer. Ascorbic acid was taken as a standard.
The total scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging (\%)} = \{A_{control} - A_{sample}/A_{control} \times 100\}$$

Where, $A_{control}$ denotes absorbance of only methanol and DPPH and A_{sample} denotes absorbance of sample dissolved in methanol (Plant extract/ standard) along with DPPH.

IC_{50} value was calculated using the software Kyplot 5.0. The IC_{50} value (µg/ml) is the concentration required to inhibit 50% of the initial DPPH free radical or in simple words it gives an idea about amount of extract needed for 50% inhibition. It was calculated from the graph of inhibition curve. All the reactions were monitored in triplicate and the value were expressed as the mean ± standard deviation (S.D.).

Ferric reducing power assay

Ferric reducing power assay was done as per the protocol[20] with slight modification. The antioxidant compounds act as reducers causing the reduction of Fe^{3+}/ferricyanide complex to the ferrous form which can be monitored by determining the formation of Perl's Prussian blue at 700 nm. In a test tube 250 µl of leaf extract was taken with addition of 625 µl of sodium phosphate buffer (0.2M, pH 6.6), 625 µl of K_2 $Fe(CN)_6$ 1(% w/v) and incubated for 20 minutes at 50 °C. The tubes were then cooled and centrifuged at 3000 rpm after addition of 625 µl of TCA (10%). The upper layer of the solution or supernatant (625 µl) was mixed with equal volume of distilled water and 125 µl of $FeCl_3$ (0.1% w/v). The absorbance was finally recorded at 700 nm. Higher absorbance value indicated higher reducing power.

Quantitative estimation of total flavonoids

The total flavonoids were estimated quantitatively using $AlCl_3$ method with some modifications[21]. A total of 250 µl of sample was taken in a test tube to which 750 µl of deionized water and 75 µl of 5% $NaNO_2$ was added. Following incubation for five minutes at room temperature, 150 µl of 10% $AlCl_3$ was added. It was then incubated for six minutes at room temperature followed by addition of 500µl of 1 mM NaOH and 275 µl of deionized water. It was then incubated for 30 minutes at room temperature. The absorbance of the yellowish orange color produced by interaction of flavonoid with $AlCl_3$ was recorded at 510 nm using spectrophotometer. Quercetin was taken as a standard and the total flavonoid content was calculated by taking reference from a calibration curve (y = 0.2071x - 0.2048) of quercetin taken at different concentrations (1-5 mg/ml) [Supplementary Figure 1(a)]. The total flavonoids were thus expressed as mg of quercetin equivalent per g of extract i.e., mg QE/ g. Data was expressed as mean of triplicates ± standard deviation.

Quantitative estimation of total phenol

The total phenolic content of the sample was determined using the Folin – Ciocalteu method[22] with slight modification. 100 µl samples were taken in a test tube and to it 400 µl of 10 % Folin reagent was added (1 ml Folin + 9 ml distilled water). The mixture was incubated in dark for 5 minutes at room temperature followed by addition of 1 ml of 5% Na_2CO_3. After incubating it for 2 h in dark at room temperature, absorbance was recorded at 730 nm using spectrophotometer. Gallic acid was taken as a standard and the total phenol content was calculated by taking reference from a calibration curve (y = 0.0075x - 0.0252) of gallic acid [Supplementary Figure 1(b)] taken at different concentrations (50-300 µg/ml). The total phenols in the extract were thus expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g). Data was expressed as mean of triplicates ± standard deviation.

Antimicrobial activity

Antimicrobial activity of the acetone and methanol extracts of different tea clones was carried by agar well diffusion method[23-25]. Four different bacterial strains were employed out of which two were gram positive bacteria (*Staphylococcus* sp. and *Bacillus* sp.) and the other two were gram negative bacteria (*Escherichia coli* and *Klebsiella* sp.).

Agar well diffusion method

Sterile petri plates of 90 mm were taken to which 25 ml of MHA (Muller Hinton agar) media was poured under aseptic conditions. After the solidification of the media 45 µl of bacterial strains was pipetted into each plate and swabbed or spread uniformly using a sterile cotton swab. A well was punctured into the solidified agar using a sterile micro tip. To each agar wells 100 µl of plant extracts (10 mg/ml) was pipetted along with a sterility control (DMSO) and a positive control (Streptomycin sulphate: a broad spectrum antibiotic used as a standard). The concentration of the standard taken was 10 folds lower than the acetone and methanol extracts. The experimental set up was same for all the samples which were performed in duplicates. The plates were incubated at 37°C for 24 hours or 48 hours if needed. The antimicrobial activity was recorded with reference to appearance of clear halo or inhibition zone on the plates.

MIC (Minimal Inhibitory Concentration) determination

The MIC value was further determined only for *Staphylococcus* sp. using agar well diffusion technique as mentioned above. Different concentrations were taken i.e., 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml, 4 mg/ml, 8 mg/ml and 10 mg/ml respectively. 100 µl of each dilution rendering different concentrations was introduced into the wells of MHA (Muller Hinton Agar) plates in duplicates which were pre-inoculated with *Staphylococcus* sp. where DMSO was used as a control. The least concentration of the acetone or methanol extract of sample, showing a clear zone of inhibition was considered as its MIC[25].

GC-MS analysis

The acetone extracts of the sample S1 (TS-569) and S3 (AV2) was further employed for GC-MS analysis. The GC-MS analysis was performed using a GCMS-QP2010 Plus (Shimadzu Corporation, Kyoto Japan) the details of which are discussed in previous reports[26].

***In-silico* analysis**

DNA gyrase is one of the Type II topoisomerase relieving strain while ds DNA is unwounded by RNA polymerase or by helicase specifically at replication fork. It either introduces negative supercoiling or relaxes the positive supercoiling and thus help in the initiation and progression of DNA replication[27]. Hence gyrase may be regarded as one of the major house-keeping proteins among organisms. This fact has been used in the docking study. Crystal structure of *S. aureus* DNA gyrase (pdb id: 5CDN) was downloaded from PDB database (http://www.rcsb.org/structure/5CDN). The resolution of the structure was 2.79 Å and was obtained through X-ray diffraction method. This crude structure was polished by removal of water and addition of polar hydrogen bonds. The dimensions of grid box were taken in such a way that the whole protein could be considered for searching the best binding site and finally the protein structure was ready for docking. The structure of Phenol was downloaded from NCBI Pubchem database. The physiochemical properties of phenol were taken into consideration. Molecular docking was done in Auto dock Vina software and was visualized via Pymol software.

Statistics

The readings were taken in triplicates and data was represented as mean ± sd. Statistical analysis relating to t- test for paired comparison of mean was performed employing KyPlot software (version 5.0). Results were considered significant at level of significance $p \leq 0.001$.

RESULTS

Qualitative test

Acetone, methanol, and ethanol persistently proved to be the most potent solvent for various phytochemical extractions as it gave positive result for tests like flavonoid, tannin, steroid, diterpenes, terpenoids, coumarin, cardiac glycoside, saponin and reducing sugar (Figure 1). The result varied to some extent in different clones and solvents since traces of phytochemicals named above were also found in extracts prepared from, diethylether, ethylacetate, and cold water as shown in the heatmap (Figure 1). However traces of phytochemicals like cardiac glycoside, flavonoid and tannin were found in extracts of less polar solvents like benzene, chloroform and hexane of seed clones S1 and S2.

The antioxidant ability of the sample cannot be concluded only by one method[28], so we used methods to estimate total phenols and flavonoids quantitatively, and also performed DPPH free radical scavenging assay and FRP assay.

DPPH assay

The highest percentage of radical scavenging (Supplementary Figure 2) at single concentration 200 µg/ml was recorded in acetone extracts (200 µg/ml) i.e. 71.90% followed by methanol (75.31%) and ethanol (73.90%) against ascorbic acid used as standard (94.82%). Overall, the lowest scavenging activity was seen in benzene extracts. Acetone and methanol being the best solvents to extract compounds with greater antioxidant potential was further compared with ascorbic acid (standard) at various concentrations ranging from 1 mg/ml to 5 mg/ml. Both the solvent extracts i.e. acetone Figure 2(a) and methanol Figure 2(b) showed antioxidant potential or rather free radical scavenging activity at par with the standard (ascorbic acid). However, acetone gave more promising result comparatively in almost all clones. Regarding samples, acetone extracts of S1 and methanol extracts of S2 showed better antioxidant potential compared to other clones with lowest IC_{50} value being 0.111 ± 0.001 mg/ml and 0.478 ± 0.028 mg/ml respectively. The IC_{50} value of standard (ascorbic acid) was recorded as 0.057 ± 0.000 mg/ml (Table 1).

Ferric reducing power assay

Similarly, acetone extracts Figure 2(c) showed higher ferric reducing power in almost all the clones than in methanol Figure 2(d) with S1 and S2 mostly giving better results. The ferric reducing power was almost at par with the ferric reducing power of ascorbic acid used as standard.

Total Phenol

Acetone extracts gave the best result overall along with the highest value of phenol recorded in acetone extracts of China seed variety (S2) as 37.77 ± 1.28 mg GAE/g. Next to acetone, methanol and ethanol gave persistently better results (Table 2). The lowest value recorded was that of chloroform extracts of S2 i.e. 0.25 mg GAE/g of total phenols.

Total flavonoid

The highest value of 722.94 ± 127.01 mg QE/g was recorded for methanolic extracts of S1 (Table 3). The lowest value was recorded for hexane extracts of S3 as 399.42 ± 2.73 mg QE/g.

Antimicrobial screening

From the antimicrobial activity studied, acetone and methanol extracts were found to be more effective and bactericidal against *Staphylococcus* sp. Figure 3 (1) and on the other hand minimum or negligible activity was observed against other bacterial strains. When compared with the standard, pipetting more extracts into the well proved beneficial as the volumetric increase of plant extracts gave results almost at par with the 100 µl of the standard Figure 3(2). The MIC of the acetone extracts of tea clones was found to 4 mg/ml Figure 4 (1) whereas for methanol extracts the MIC was recorded as 8 mg/ml Figure 4 (2).

Gas chromatography- mass spectrometry analysis

Further analysis of the extracts using Gas chromatography- mass spectrometry identified bioactive compounds like Phenol, 3,5-bis(1,1-dimethylethyl), caffeine and Vitamin E as the probable compounds as antibacterial agents in both the extracts of S1

(Supplementary Figure 3 ; Supplementary Table 3) and S3 (Supplementary Figure 4; Supplementary Table 4).

***In-silico* results**

The binding affinity of phenol to 5CDN was found to be -7.2 kcal/mol which showed the significant effect of phenol with the gyrase protein (Figure 5). The other two compounds did not show any effective binding.

DISCUSSION

The nature of extracting solvent plays an important role in extraction of potential compounds of antioxidant activity since the compounds differ in chemical characteristics, polarities and solubilities[29]. Presence of alkaloids, flavonoids, saponins, terpenoids and phenols were reported in plant extracts of *Camellia sinensis* (purple tea) and the solvents with higher polarity i.e. water, ethanol and acetone were found to extract major phytochemicals groups than non-polar ethyl acetate and chloroform[30]. Methanol showed better extraction properties than acetone and ethyl acetate for extracting few phytochemicals like flavonoid, tannin, triterpenes, lipid and reducing sugar in black packaged tea where other solvents showed minimum activity[28]. The polar solvents and in some cases even the least polar solvents showed best result in extracting phytochemicals from fresh leaves of seed clones like S1 and S2 and we could therefore infer that in addition to extraction of samples using solvent with different polarities, extraction time and procedure, the state of sample also matters in phytochemical extraction since the phytochemical constituent slowly degenerates from the time of plucking up to manufacturing. Qualitative screening of phytochemicals is important to the pharmaceutical industry since the presence of a phytochemical of interest may lead to its further isolation, purification and characterization[31].

Table 1: **IC50 value of acetone and methanol extracts prepared in different concentrations (mg/ml) during DPPH Assay**

Accession	IC_{50} (mean ± sd) of acetone extracts (mg/ml)	IC_{50} (mean ± sd) of methanol extracts (mg/ml)
S1 (TS569)	0.111 ± 0.001	0.635 ± 0.028
S2 (China)	0.159 ± 0.002	0.478 ± 0.028
S3 (AV2)	0.600 ± 0.002	1.030 ± 0.061
S4 (PS312)	0.294 ± 0.008	1.595 ± 0.297
S5 (Assam)	0.515 ± 0.002	1.016 ± 0.039
ST (Ascorbic acid)	0.057 ± 0.000	0.056 ± 0.000

Data expressed as means of triplicates ± sd

Table 2: Determination of total phenol content (TPC) expressed as mg Gallic acid equivalent (GAE) /g in fresh leaves tea clones extracted by different solvents

Solvents	S1 Mean ± sd (mgGAE/g)	S2 Mean ± sd (mgGAE/g)	S3 Mean ± sd (mgGAE/g)	S4 Mean ± sd (mgGAE/g)	S5 Mean ± sd (mgGAE/g)
Hexane	1.61 ± 0.08	0.60 ± 0.75	3.66 ± 3.51	1.00 ± 0.04	1.08 ± 0.38
Benzene	2.14 ± 0.98	0.38 ± 0.00	2.86 ± 0.79	1.21 ± 0.04	1.50 ± 0.08
Chloroform	1.77 ± 0.08	0.25 ± 0.19	1.50 ± 0.00	1.45 ± 0.08	1.85 ± 0.26
Diethylether	3.42 ± 0.15	7.18 ± 0.72	2.49 ± 0.26	2.14 ± 0.00	3.34 ± 0.11
Ethylacetate	6.41 ± 0.15	5.40 ± 0.68	3.77 ± 0.11	3.69 ± 0.08	5.42 ± 0.26
Acetone	32.44 ± 0.98	37.77 ± 1.28	32.06 ± 0.75	14.57 ± 1.66	18.65 ± 2.07
Ethanol	18.25 ± 0.15	16.92 ± 0.98	17.88 ± 0.60	14.25 ± 4.75	13.72 ± 0.08
Methanol	32.89 ± 7.96	31.50 ± 9.99	23.82 ± 6.45	10.73 ± 0.15	11.50 ± 1.24
Water	4.04 ± 0.19	1.53 ± 0.11	2.36 ± 0.53	2.52 ± 0.38	2.25 ± 0.30

$y = 0.0075x-0.0252$, $R^2 = 0.9864$. Data expressed as means of triplicates ± sd

Table 3: Determination of total flavonoid content (TFC) expressed as mg Quercetin equivalent (QE)/g in fresh leaves of tea clones extracted by different solvents

Solvents	S1 Mean ± sd (mgQE/g)	S2 Mean ± sd (mgQE/g)	S3 Mean ± sd (mgQE/g)	S4 Mean ± sd (mgQE/g)	S5 Mean ± sd (mgQE/g)
Hexane	412.94 ± 8.19	446.74 ± 23.22	399.42 ± 2.73	418.73 ± 13.66	537.52 ± 28.68
Benzene	406.18 ± 1.37	441.91 ± 24.58	488.27 ± 73.75	401.35 ± 0.00	463.16 ± 5.46
Chloroform	428.39 ± 2.73	482.47 ± 19.12	412.94 ± 2.73	467.02 ± 10.93	477.64 ± 15.02
Diethyl ether	467.99 ± 12.29	473.78 ± 36.87	433.22 ± 1.37	448.67 ± 4.10	561.66 ± 16.39
Ethyl acetate	470.88 ± 5.46	507.58 ± 10.93	433.22 ± 4.10	424.53 ± 8.19	469.92 ± 23.22
Acetone	513.76 ± 136.03	675.62 ± 51.90	617.67 ± 32.78	579.04 ± 8.19	669.82 ± 87.41
Ethanol	528.83 ± 19.12	557.80 ± 8.19	570.35 ± 6.83	506.62 ± 9.56	611.88 ± 16.39
Methanol	722.94 ± 127.01	581.94 ± 17.75	545.24 ± 45.07	519.17 ± 35.51	574.22 ± 1.37
Water	424.53 ± 10.93	406.18 ± 6.83	432.25 ± 10.933	423.56 ± 34.14	431.29 ± 4.10

$y = 0.2071x - 0.2048$, $R^2 = 0.9625$. Data expressed as means of triplicates ± sd

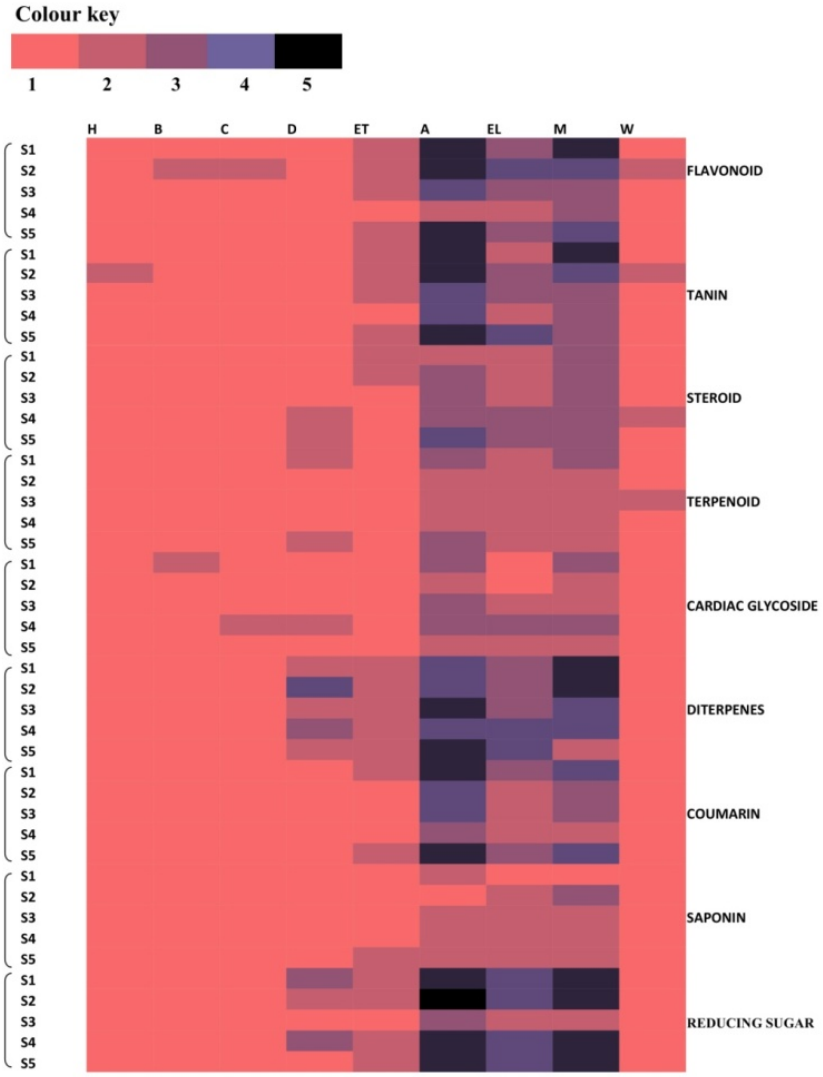

Figure 1: Heat map representing the qualitative phytochemical profiling of different extracts of selected tea clones
Colour key provided from pink to black represents the intensity in increasing order. H-Hexane, B-Benzene, C-Chloroform, D-Diethyl ether, ETEthyl acetate, A-Acetone. EL-Ethanol, M-Methanol, W-Water

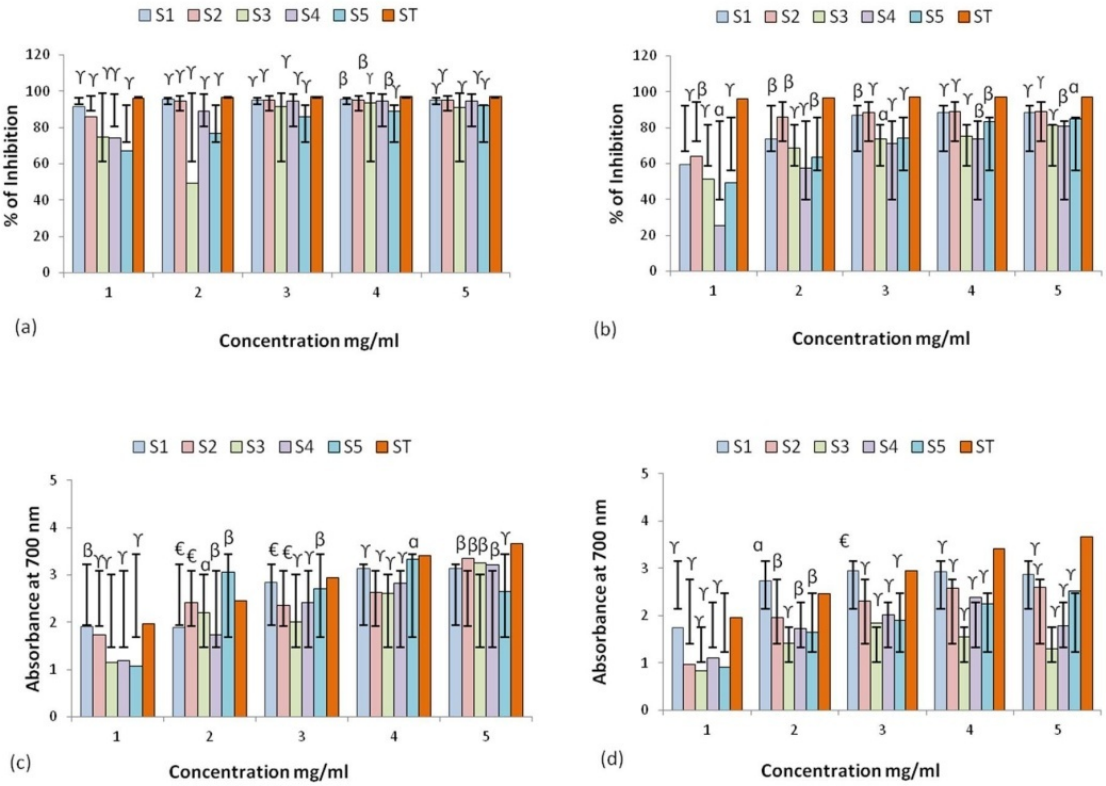

Figure 2(a): DPPH scavenging activity of acetone extracts; (b): DPPH scavenging activity of methanol extracts; (c): Ferric reducing power of acetone extracts (d): Ferric reducing power of methanol extracts;
$^{\alpha}p < 0.05$; $^{\beta}p < 0.01$; $^{\Upsilon}p < 0.001$; $^{\epsilon}$non significant

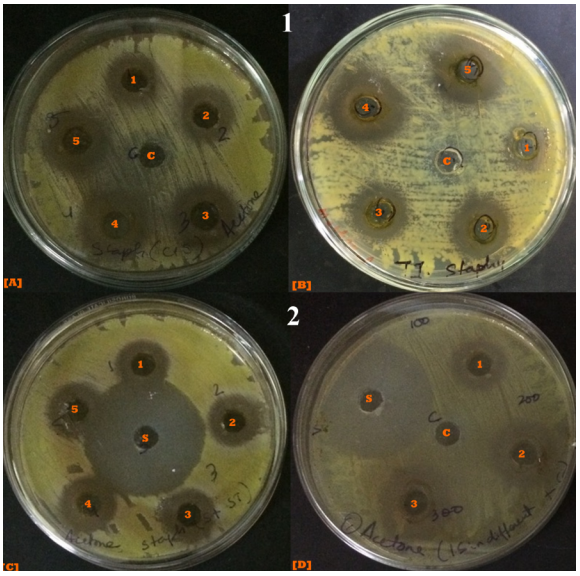

Figure 3(1): Antimicrobial activity of tea extracts against *Staphylococcus* sp.
(A) Acetone (B) Methanol, 1-5 different tea clones; C sterility control (DMSO); (2) Antimicrobial activity of tea extracts against *Staphylococcus* sp. (C) Acetone extract (100 µl of 10 mg/ml) of five different tea clones with standard; 1-5 different five clones; S positive control (Streptomycin sulphate), (D) Acetone extract of S1 with standard; 1-3 different volumetric dose; 1– 100 µl , 2– 200 µl, 3– 300 µl, S positive control (Streptomycin sulphate); C sterility control DMSO

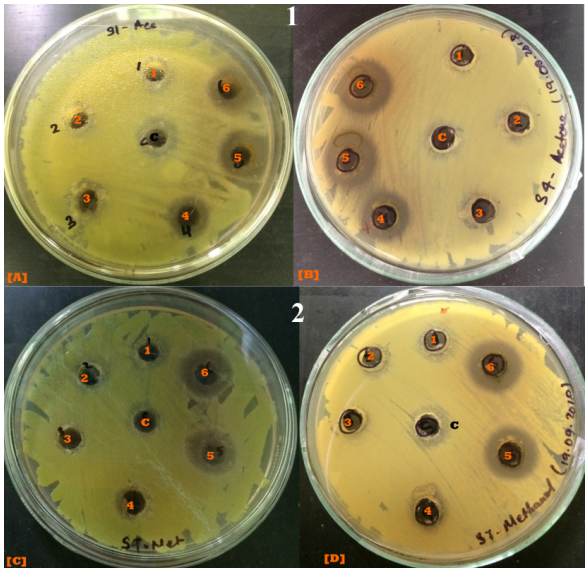

Figure 4(1): MIC value determination from antimicrobial activity of tea extracts against *Staphylococcus* sp. using agar well diffusion method (A) Acetone extracts of S2 (B) Acetone extracts of S4; 1-5 different concentrations 1-0.25 mg/ml, 2– 05 mg/ml, 3– 1 mg/ml, 4– 4 mg/ml, 5– 8 mg/m, 6– 10 mg/ml; C sterility control (DMSO); (2) MIC value determination from antimicrobial activity of tea extracts against *Staphylococcus* sp. using agar well diffusion method (C) Methanol extracts of S4(D) Methanol extracts of S7; 1-5 different concentrations 1-0.25 mg/ml, 2– 05 mg/ml, 3– 1 mg/ml, 4– 4 mg/ml, 5– 8 mg/m, 6– 10 mg/ml; C sterility control DMSO

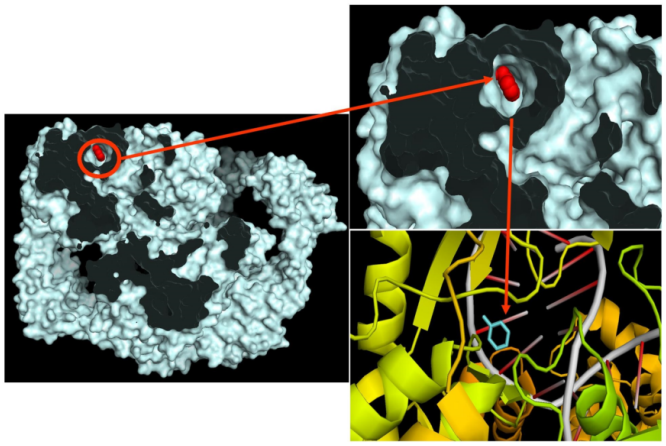

Figure 5: *In-silico* docking of *S. aureus* DNA gyrase (pdb id: 5CDN) showing significant binding affinity -7.2 kcal/mol with phenol

Methanol[28] ethanol and acetone extract[14] has already been reported to have strong antioxidant property in black tea. Previous report[14] highlights the varying antioxidant activity with changes in percent concentration of the solvent thus reporting 50% ethanol and 50% acetone with maximum antioxidant potential in mate tea and black tea respectively and also showed the capability of hot water showing moderate antioxidant potential in black tea and higher antioxidant potential in mate tea when compared to other 100% solvent. Thus it could be inferred that solvent potential may be enhanced or reduced with altering the percent concentration of the solvent.

The IC_{50} value calculated represents the exact concentration for 0.5 absorbance at 700 nm and the reducing power mostly increases with increasing concentration of antioxidant compounds[28,32,33]. Previous report on fresh tea leaf sample[34] has stated methanol extract showing highest reducing power based on state of sample where greater ferric reducing power was showed by shoot extract followed by young leaves and mature leaves.

The minimum level of phenols was recorded in less polar solvent extracts like hexane, benzene, and chloroform. However, the consistency of better result did not limit to the range of polarity in increasing or decreasing order. It varied among the different sample as well as solvents. It has already been reported in previous works that green tea was a richer source of phenolics than white tea and found 40% aqueous ethanol to be useful for extracting catechin[15]. Total phenol content estimation following Folin-Ciocalteu reagent method showed 50% acetone, 50% N, N-dimethylformamide (DMF), 50% ethanol and 50% methanol to be suitable for extracting total phenols[14].

Previously aqueous tea extracts were found to be effective against range of bacterial strains including *Staphylococcus aureus* with bactericidal activity against *Staphylococcus aureus* and *Yersinia enterocolitica*[35]. However in our study significant antimicrobial activity was found for acetone and methanol extract against *Staphylococcus* sp. with minimum or negligible activity against other strains.

Vitamin E being a powerful antioxidant showing scavenging as well as anti-inflammatory properties was also found to be effective against gram positive bacteria than gram negative ones owing to the fact of lipopolysaccharides present in their outer membrane[36]. On the other hand, the antimicrobial activity of *Arabica coffee* extracts against *Staphylococcus epidermidis* and *Enterococcus faecalis* was found to be independent from caffeine content[37]. However, caffeine was found to potentiate or enhance the antibacterial activity of amoxicillin against *Staphylococcus aureus*[38]. Significant inhibition of hexane extract of *Ulva reticulata* against *Staphylococcus aureus* and *Escherichia coli* reported presence of phenol, 3, 5-bis (1, 1-dimethylethyl) in GC-MS analysis as the probable compound giving antibacterial activity[39].

Phenol is a well-known antimicrobial agent with both bacteriostatic and bactericidal effect. Phenol may exert its effect on external membrane of bacterial cell wall, cytoplasmic membrane, organelles, cytosol as well as on spores[40]. The penetrating power of phenol into organic matter is quite high and active phenol may enter through bacterial cytoplasmic membrane via passive diffusion and active transport. Moreover, phenol acts as strong oxidizing agent and converts akin to oxide and gas, which ultimately destabilize the spores[40]. Thus, the binding of phenol to gyrase a protein may also exert as strong antimicrobial effect.

CONCLUSION

Acetone, methanol, and ethanol showed persistent and better results to extract various phytochemicals like flavonoid, tannin, steroid, diterpenes, terpenoids, coumarin, cardiac glycoside, saponin, and reducing sugar. Elaborate qualitative phytochemical profiling thus gave an idea about the potency of particular solvent in extracting specific compound of interest. Acetone extracts gave the best result overall during radical scavenging assay, ferric reducing power assay, total phenol and flavonoid estimation followed by methanol. Out of the four bacterial strain studied the acetone extracts along with methanol extracts was found effective against the *Staphylococcus* sp. with the MIC value 4 mg/ml for acetone extracts and 8 mg/ml for methanol extracts respectively. GC-MS and *in- silico* analysis suggested phenol 3, 5-bis (1, 1-dimethylethyl) as the potent antimicrobial compound. Thus, it can be concluded that abundancy of phytochemicals may vary from one clone to the other or its state (either fresh, dry or manufactured), beside varying time and procedure and the type of solvent utilized for extraction. We could also infer from our results that there isn't any pattern of potentiality of solvents in increasing or decreasing order. The collective information of this work could promote future research to utilize resources in an efficient manner.

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