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Full Length Original Research Paper

Evaluation of antioxidant, anti-inflammatory and antimicrobial activities of two different extracts of *Camellia sinensis* (L.) O. Kuntze

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Abstract: Background: White tea is the mildest tea made from new young leaves of the tea plant *Camellia sinensis* (L.) O. Kuntze belonging to the family Theaceae. Objective: To compare the efficacy for phytochemical, antimicrobial, anti-inflammatory and antioxidant activities of methanol and acetone extracts of White tea. Methodology: The methanolic and acetone extracts of the white tea leaves were screened for all pharmacological activities. The four bacterial strains and one fungal species were investigated for antimicrobial activity of white tea. Phytochemical screening, Non-enzymatic and enzymatic antioxidant activity was analysed by standard methods. The prevention of hypotonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity. The compounds were identified by GC-MS analysis. Results: The methanolic extracts showed higher activity against four of the bacterial organisms and one fungus than acetone extracts. The methanolic extract of white tea has a high level of total phenolic content and reducing power than acetone extracts. Antioxidant activity of the methanolic extracts of white tea was higher than acetone extracts for all methods expect by Ferric reducing antioxidant power methods. It showed anti-inflammatory activity in terms of percent prevention of lysis of 40% and 38% for 100µl of methanolic and acetone extracts. The compounds present in both extracts were identified using GC-MS analysis. Conclusions: The results of the present study indicate compounds isolated from methanolic and acetone extracts of White tea possesses pharmacological properties. Therefore, they could be used as natural potential compounds of natural origin for pharmaceuticals plant-based products industry.

Keywords: Antioxidant activity, Anti-inflammatory activity, Antimicrobial screening, GC-MS analysis, Phytochemicals, White tea extract.

Introduction

Plants are the potential source of natural antioxidants and produce antioxidative compounds in order to survive and counteract with reactive oxygen species. Extracts derived from tea extracts play an important role in both traditional and modern medicine [1]. Antioxidants are dietary substance that protects body cells from the oxidative damage to a target molecules caused by oxidation from free radicals by reactive oxygen species [2]. Free radicals are the main culprit in lipid peroxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources [3]. Though many synthetic antioxidants are available they possess some side effects and toxic property on human health. Natural antioxidants, particularly in fruits, vegetables and beverages have gained interest among consumers [4].

Industrialization has led to the emission of various pollutants which has become a cause for induction of cancer, asthma and cardiovascular diseases. Antioxidants lower the risk of free radicals mediated deadly diseases [5]. During the last two decades there has been in search for new plant derived drugs containing the medically useful

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alkaloids, glycosides, polyphenolics, steroids and terpenoids derivatives, which contributes to the antioxidant property. The traditional system of medicine also claims that medicinal plants can be used as antibacterial, antifungal and anti-inflammatory agents. Inflammation is a process involved in the pathogenesis of several disorders like arthritis and cardio vascular disease [6]. The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [7]. During the acute phase of inflammation processes, polymorpho-nuclear neutrophils and monocytes react to chemotactic agents by entering the inflammatory focus and ingest and digest the foreign material [8]. The side effects of the anti-inflammatory drugs pose a major problem during their clinical uses so research is increasing in this area for the development of anti-inflammatory drugs with lesser side effects.

Most widely consumed beverage in the world is *Camellia sinensis* (L.) O. Kuntze belonging to the family Theaceae [9]. White tea is obtained from new growing buds and young leaves of the tea plant. White tea retains the high concentrations of catechins that are present in fresh tea leaves [10]. The little buds that form on the plant are covered with silver hairs that give the young leaves a white appearance for white tea. Therefore, the use of natural antioxidants, plant phytochemicals is important in less damaging to human health and environment. To best of our knowledge, the scientific community is behind natural compounds in search of antioxidant, which insisted us in finding out the antimicrobial, antioxidant and anti-inflammatory activity of white tea which is not yet explored when compared to green and black tea. Therefore the main objectives of this study are to assess all pharmacological activities of white tea extracts and to determine the main active components using GC-MS analysis.

Methods and Materials

Plant material

White tea was collected from Highfield estate, Connoor, Niligiri district, Coimbatore, Tamil Nadu, India. The leaves were collected, shade dried and coarsely powdered by standard method of Harborne (1998) [11] and stored in an airtight container for further work.

Preparation of extract

The leaves were dried under shade dired and powdered well in a mixture grinder. The methanolic and acetone extracts of the active ingredient of white tea leaves were carried out using the method as described by Harborne (1998) [11]. 5 g powder of plant material used for the extraction. Powdered material extract with 150 ml of ethanol in Soxhlet apparatus separately for 3 h. Then ethanol filtrate was concentrated separately on water bath to a thick paste and dried under rotary evaporator. The residue was weighed to give the yield of 0.8 g and stored at 4°C in cold room.

Chemicals

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Merck Chemical Supplies (Damstadt, Germany), SD fine (Mumbai, India) and Hi Media (Mumbai, India). All other chemicals used were obtained commercially and were of analytical grade.

Phytochemical Analysis

Qualitative analysis of phytochemicals

Preliminary phytochemical analysis was carried out using standard analytical procedure

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provided by Odebiyi and Ramstard (1978) [12] to investigate the presence or absence of secondary metabolites.

Quantitative determination of phytochemicals

Determination of total phenolics content

The method according to the literature of Singleton and Rossi (1965) [13] with little modification by using Folin-Ciocalteu reagent was used to determine total phenolics content. 0.5 mL of the extract (1 mg/mL) was mixed with 0.5 mL of 10% Folin-Ciocalteu and was left to stand at room temperature for 1 min. 2.5 mL of sodium carbonate solution was added sequentially in each tube. The resulting mixture was vortexed for 15 sec and incubated at room temperature in the dark at 40°C for 30 min for colour development. Reagent blank using distilled water was prepared. The absorbance of total phenolics was measured at 725 nm against the reagent blank using a spectrophotometer. Total phenolics content was expressed as mg/g Gallic acid equivalent (GA). The experiment was conducted in triplicate and the results were expressed as mean ± SD values.

Determination of reducing power

Reducing power was determined by Siddhuraju, Mohan and Becker method (2002) [14]. 20-100 µg of extracts in 1 ml of phosphate buffer with 5 ml of 0.2 M phosphate buffer (pH 6.6) and 5 ml of 1% potassium ferricyanide solution were incubated at 50°C for 20 minutes. After the incubation, 5 ml of 10% Tricarboxylic acid was added. The content was then centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 ml) was mixed with 5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. Then the absorbance of the reaction mixture was read spectroscopically at 700 nm. All tests were carried out in triplicate.

Antimicrobial screening

Antimicrobial screening

The bacterial and fungal test organisms wer obtained from Department of Biotechnology Kongunadu arts and science college, Coimbatore The antimicrobial activity of white tea extracts wa evaluated using four bacterial strains (*Proteu mirabilis, Salmonella typhi, Escherichia coli* an *Bacillus subtili)* and fungal strains (*Candid albicans*). The cultures were maintained in th Nutrient Agar (NA) and Potato Dextrose Agar (PDA at 4 °C and subcultured before use.

Antimicrobial assay Bacterial and Fungal Strains

Antimicrobial activity of white tea extracts were tested using agar well diffusion method according to Raghu and Ravindra (2010) [15] and was compared with commercial antibiotics viz., Gentamycin and Chloramphenical. The antibacterial and antifungal activity of white tea extracts were determined by measuring zone of inhibition. A swab of the bacterial and fungal suspension containing 1×10^8 CFU/ml was spread onto petriplates containing Nutrient agar and Sabouraud dextrose agar respectively. The bacterial were first grown in nutrient agar for 18 h before use. The spread plates were prepared in potato dextrose agar using 0.1ml of inoculums containing appropriate fungal strains of 20 hours culture. The inoculum suspensions were standardized and then tested against the effect of the white tea extracts at the concentration of 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml. The plates were incubated overnight at 37°C±0.5°C for 24 hours after which they were observed for zones of inhibition. The effects were compared with that of the standard antibiotic viz., gentamycin and chloramphenical at a

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concentration of 1000µg/ml. All tests were carried out in triplicate manner under sterile conditions.

Determination of antioxidant Activity

DPPH radical scavenging activity

The DPPH assay was done according to the method of Brand-Williams (1995) [16] with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100ml methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10mlstock solution with 45 ml methanol to obtain an absorbance at 515 nm using the spectrophotometer. White tea extracts (150 ml) were allowed to react with 2850 ml of the DPPH solution for 24 h in the dark. Then the absorbance was read at 515 nm. Results were expressed in mM TE/g fresh mass. All tests were run in triplicate and averaged. Percentage free radical scavenging activity was calculated using the formula

$$\text{Scavenging activity (\% Inhibition)} = \frac{(OD\ of\ control) - (OD\ of\ test\ sample)}{OD\ of\ control} * 100$$

ABTS radical cation scavenging activity (ABTS)

The ABTS assay was done according to the method of Arnao et al. (2001) [17] with some modifications. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium per sulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1ml ABTS solution with 60 ml methanol to obtain an absorbance at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. White tea extracts (150 ml) were allowed to react with 2850 ml of the ABTS solution for 2 hours in a dark condition. Then the absorbance was read at 734 nm using the spectrophotometer. Results were expressed in mM Trolox equivalents (TE)/g fresh mass. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve. All tests were carried out in triplicate. Percentage ABTS radical cation scavenging activity was calculated using the scavenging activity formula as presented earlier.

Ferric reducing antioxidant activity (FRAP)

The FRAP assay was done according to Benzie and Strain method (1999) [18] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2$.$3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$.$6H_2O$ solution. 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°C before use. White tea extracts (150ml) were allowed to react with 2850 ml of the FRAP solution for 30 minutes in the dark condition. The colored product [ferrous tripyridyl triazine complex] was read at 593 nm. Results were expressed in mM TE/g fresh mass. All tests were run in triplicate and averaged. Percentage ferric reducing scavenging activity was calculated using the scavenging activity formula as presented earlier.

Oxygen radical absorbance capacity (ORAC)

The ORAC procedure used an automated plate reader (KC_4, Bio Tek, and USA) with 96-well plates [19]. Analyses were conducted in phosphate buffer pH 7.4 at 37°C. Peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. Results were expressed as mM TE/g fresh mass. All

tests were run in triplicate and averaged. Percentage oxygen radical scavenging activity was calculated using the scavenging activity formula as presented earlier.

Superoxide dismutase scavenging activity

Superoxide dismutase activity was estimated spectrophotometrically following the method of Kakkar et al. (1984) [20]. One unit of SOD activity was defined as the amount of enzyme that inhibits the rate of reaction by 50%under specified conditions. The enzyme activity was expressed as units/g dry tissue. The reaction mixture consisted of (10- 1000µg) dilutions of plant extract made to 1ml with distilled water, 1ml, 60µM PMS, in phosphate buffer (0.1M, pH7.4) and 150µM, 1ml NBT in phosphate buffer. Incubation at ambient temperature followed for 5minutes, and the resultant colour was read spectrophotometrically at 560nm against a blank. The effect of BHA was also determined by replacing plant extract with 1ml BHA (10-1000µg) in methanol in the reaction mixture. All tests were run in triplicate and averaged. Percentage SOD scavenging activity was calculated using the scavenging activity formula as presented earlier.

Nitric oxide scavenging activity

The Nitric oxide radical inhibition was done according to Badami et al. (2003) [21]. 0.5ml (10–50 µg/ml) of both extracts and 2 ml Sodium Nitroprusside (10 mM), 0.5 ml Phosphate Buffer Saline was kept at 25°C for 150 min. Then 1 ml of Sulfanilic acid reagent (0.33% Sulfanilic acid in 20% glacial acetic acid) was added to 0.5 ml of reaction mixture for 5 min. 1 ml NEDD was added and allowed to stand for 30 min at 25°C. The absorbance was measured at 540 nm and it was done thrice. The activity was compared with ascorbic acid, which was considered as standard antioxidant.

Anti-inflammatory activity

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity [8]. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilised Alsever solution (2% dextrose,0.8% sodium citrate,0.5% citric acid and 0.42% sodium chloride in water).The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%, pH 7.2)and 10% (v/v) suspension was made with isosaline. Add 1 ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Diclophenac sodium was used as reference drug. Instead of hyposaline 2 ml of distilled water was used as the control. All the assay mixture were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using UV spectrophotometer at 560 nm. All tests were performed in triplicate. The percentage haemolysis was calculated by assuming the hemolysis produced in presence of distilled water as 100%. The percentage of haemolysis was calculated using the formula

$$\% \text{ Inhibition of Haemolysis} = \frac{(OD \text{ of control}) - (OD \text{ of test sample})}{OD \text{ of control}} \times 100$$

Gas Chromatography-Mass Spectroscopy analysis (GC-MS)

The methanolic and acetone extract of the white tea was analysed by GC-MS analysis. The GC column dimension was 30 x 0.25 mm x 2.0 mm A13-35 MS fused silica capillary column. The condition maintained were, the injector temperature was about 250°C, column temperature isothermal at 100°C, then programmed to rise upto 250°C at 6°C/minutes and be held at this temperature for 10 minutes. The ion source temperature was 200°C and the

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interface temperature is 240°C. Helium gas was engaged as a carrier gas at the rate of 1 ml/minutes. The spectra were obtained in the EI mode with 700x ionization energy. The compounds were identified by comparing with standard of the mass spectra and matched with inbuilt library.

RESULTS AND DISCUSSION

Phytochemicals Analysis

Phytochemicals are bioactive non-nutrient chemical compounds found in plant, fruit, vegetables and grains. Some of the well-known phytochemicals are flavonoids, phenolic compounds, lycopenes and carotenoids. It may act as antioxidant, anti-microbial, enzyme stimulant, hormone analogs and also as novel source of drugs. Plant has a potential to be used as a source of natural antioxidant and also as a food supplement [22]. The Phytochemicals analysis of methanol and acetone extracts were tabulated in Table1. In particular, methanolic extract showed a positive result for tannins and saponins and negative result for the alkaloids when compared with acetone extract. Further phytochemical analysis of methanolic and acetone extracts revealed that the antimicrobial activity is due to the presence of phenolic compounds. Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect.

Medicinally, this is important for the treatment of inflamed or ulcerated tissues. Tannins have important roles such as stable and potent antioxidants [23]. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis.Plants containing phenols and flavonoids have been reported to possess strong antioxidant properties. Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plant is alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications.

Determination of total phenolics content

Recovery percentage yield of white tea was found to be 40% and 30% in methanolic and acetone extracts. Total phenolic content of white tea was found to be 0.92 ± 0.02 and 0.54± 0.02 for methanolic and acetone extracts. The methanolic extract was found to be containing high level of extract recovery percentage and total phenolic content than acetone extract. The major contributors of phenolics have showed a positive result in the qualitative analysis of the methanolic extract. The acetone extract showed a negative result for tannins and saponins which might be a cause for the decrease in the phenolic content [24]. Polyphenols are the major plant compounds with antioxidants activity due to their redox properties, it play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet O_2 or decomposing peroxides.The antioxidant property of the compounds was well correlated with the content of their phenolic compounds. Phenols contain good antioxidant, antimutagenic and anticancer properties [25]. Generally, phenol content may either increase or decrease in fruits and vegetables depend on the storage conditions. Most of antioxidants have been showed to be phenolic compounds and in particular flavonoids [26].Therefore, the highest content of total phenol in white tea was responsible for the better antioxidant properties.

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Determination of reducing power

The results showed that reducing power of white tea was found to be 0.32 ± 0.01 and 0.07± 0.02 for methanolic and acetone extracts. Methanolic extracts of white tea showed the highest reducing power and the values were comparable to that of phenolic content. Similar observation between the polyphenolic constituents in terms of reducing power activity have been reported for several plant extracts including tea [27]. Generally the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radicals chain through donating a hydrogen atom [28].

Antimicrobial screening

The investigation of antimicrobial activity by agar well diffusion method using four bacterial stains and one fungal strains individually showed that the methanolic and acetone extracts of white tea possess an effective anti-microbial activity against *Proteus mirabilis, Salmonella typhi, Escherichia coli, Bacillus subtilis* and *Candida albicans*. White tea extracts had shown the ability to inhibit the growth of microorganisms such as *Proteus mirabilis, Salmonella typhi, Escherichia coli, Bacillus subtilis* and *Candida albicians* at all concentrations expect 62.5µg/ml for both extracts. The methanolic extract exhibited a significant antimicrobial activity than acetone extracts. The zones of inhibition were compared with the zone formed by two standards antibiotics. The results of antimicrobial activity of white tea extracts and their zone of inhibition of antibiotics were tabulated in Table 2. This study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

Determination of antioxidant Activity

DPPH radical scavenging activity

The free radicals scavenging activity of the white tea extracts on DPPH radicals was found to have IC_{50} of 50.98 % at 250µg/ml for methanolic and was found to be 50.03 % at 300 µg/ml for acetone extract (Table 3). The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods [29]. The DPPH scavenging activity was found to be dose dependent. The strong DPPH scavenging activity of tea could be attributed in part to the tea catechins and some low molecular polyphenols. This radical scavenging activity of tea extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. Phenolic compounds and essential oils rich in phenolic compounds show potent antioxidant and DPPH radical scavenging activities [30].

ABTS radical cation scavenging activity (ABTS)

ABTS radical, a protonated radical has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals [31]. The ABTS radical cation scavenging activity of the white tea extracts at 250 µg/ml was found to be IC_{50} at 50.22 % for methanolic extract and 51.13 % at 300 µg/ml for acetone extract (Table 3), indicating that the methanolic extracts were fast and effective scavengers of the ABTS radicals. Hagerman et al. (1998)[32] have reported that the high molecular weight phenolics have more ability to quench free radicals and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group substitution than the

specific functional groups. Higher concentrations of the extracts were more effective in quenching free radicals in the system.

Ferric reducing antioxidant activity (FRAP)

FRAP activity of the white tea extracts was found to be 50.11 % for 300 µg/ml for methanolic extract and 50.98% for 250µg/ml for acetone extract (Table 3). The acetone extract showed higher ferric reducing antioxidant power activity due to low IC_{50} value. Due to the intensity of blue colour, the FRAP assay showed high changes in IC_{50} value for methanolic and low changes in acetone extract, which might be due to the presence of alkaloids [24].

Oxygen radical absorbance capacity (ORAC)

The amount of antioxidant necessary to increase the initial ORAC radical concentration by 50 % inhibition at 250µg/ml was found to be 50.62 % for methanolic and acetone extract at 300µg/ml was found to be 50.33 % respectively (Table 3). The methanolic extract showed higher ORAC values than acetone extract indicating that acetone extract has much higher O_2 scavenging activity than methanolic extract and found to be dose dependent. The oxygen free radical measures the ability of antioxidants to protect protein from damage [33]. ORAC assay provides direct measure of the hydrophilic and lipophilic chain-breaking antioxidant capacity values versus peroxyl radicals.

Superoxide dismutase scavenging activity

The investigation of results showed that superoxide scavenging activity of white tea was found to be IC_{50} at 300µg/ml 52.54 ± 0.01for methanolic extract and 51.33± 0.02 for acetone extract at 250µg/mland were tabulated in Table 3. Enzymatic antioxidants serve as an intrinsic defense tool to resist oxidative damage in plants [34]. One of the mechanisms *in vivo* is improving the endogenous cellular antioxidants mechanisms, such as up-regulation of the activity superoxide dismutase [35].

Nitric oxide scavenging activity

Nitric oxide scavenging activity of white tea was found to be IC_{50} at 300 µg/ml 50.34 ± 0.01 for methanolic extract and 51.20± 0.01 for acetone extract of white tea at 300µg/ml respectively (Table 3). Nitric oxide is generated from amino acid L-arginine by vascular endothelial cells, phagocytes and certain cells in the brain. The toxicity and damage caused by NO and O_2 is multiplied as they react to produce reactive peroxynitrite, which lead to serious toxic reactions in the biomolecules, like proteins, lipids and nucleic acids [36]. Scavengers of nitric oxide compete with oxygen and lead to the production of nitric oxide.

Anti-inflammatory activity

White tea showed anti-inflammatory activity in terms of percent prevention of lysis of 40% and 38% for 100µl of methanolic and acetone extract. The activities of all extracts were comparable to that of Diclophenac sodium at various concentrations. It exhibited higher anti-inflammatory activity in methanolic extract than acetone extract (Table 4). The lysosomal enzymes released during inflammation produced a variety of disorders. The extracellular activity of these enzymes was said to be related to acute or chronic inflammation. The diclophenac drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [8]. Since HRBC membrane similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. All the extracts of white tea showed biphasic effects

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on HRBC membrane stabilization. Lipid oxidation of blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently haemolysis. The extracts were effective against lipid oxidation in the erythrocyte membrane thus inhibiting lysis.

Gas Chromatography-Mass Spectroscopy analysis (GC-MS)

GC-MS analysis separates all phytochemicals components in a white tea extracts. GC-MS analysis showed the existence of various compounds with variable chemical structure. GC-MS analysis showed that 20 compounds were present in methanolic extract and 7 compounds in acetone extract of white tea were showed in Table 5a, 5b and Figure 1a and 1b.

Conclusion

The present study showed that the white tea extracts possess effective phytochemicals components in both extracts and strong antioxidant properties, which may be related to the amount of phenolic compounds and could serve as free radical inhibitors or scavenger or acting possibly as primary antioxidants, in addition to having their medicinal properties. This study supports the use of natural plants products as medicines because active biomolecules in white tea extracts exhibit antimicrobial activity to a considerable extent and the results strongly supports traditional use of tea plant. The methanolic extract was found to be containing high level of extract recovery percentage, total phenolic content and reducing power, antioxidant activity in all methods, anti-inflammatory and antimicrobial activity than acetone extract. The various specific compounds present in both extract of white tea were identified using GC-MS analysis. Thus, the effective source of white tea could be employed in all medicinal preparations to combat various diseases associated with oxidative stress, including cancer and related disorders.

Table 1: Phytochemicals analysis of white tea extracts

S. No	Phytochemicals	Phytochemical tests	Methanolic extract	Acetone extract
1.	Alkaloids	Wagner's test	-	+
		Mayer's test	-	+
		Hager's test	-	+
		Dragendorff's test	-	+
2.	Carbohydrates	Molish's test	+	+
		Fehling's test	+	+
		Barford's test	+	+
		Benedicts test	+	+
3.	Glycosides	Borntrager's test	+	+
		Legal's test	+	+
4.	Saponins		+	-
5.	Protein and Aminoacids	Million's test	+	+
		Biuret test	+	+
		Ninhydrin test	+	+
6.	Phytosterols	Libermann-burchard's test	+	+
7.	Fixed oils and fats	Spot test	+	+
		Saponification test	+	+
8.	Flavonoids	Alkaline reagent test	+	+
		Shionoda test	+	+
9.	Triterpenoids	Salkowski test	+	+
10.	Phenols and tannins	Ferric chloride test	+	+
		Lead acetate test	+	-

Phytochemical test: - negative and + positive

Table 2: Antimicrobial activity of white tea extracts

White tea extracts	Sample concentrations (µg/ml)	Zone of inhibition(mm)*				
		Proteus mirabilis	*S. typhi*	*E.coli*	*B.subtilis*	*Candida albicians*
control	1000	20	28	24	23	24
Methanolic	1000	17	20	15	15	17
	500	09	15	12	11	08
	250	08	10	09	08	05
	125	07	07	07	04	02
	62.5	00	04	03	00	00
Acetone	1000	12	10	11	12	13
	500	07	05	10	09	06
	250	02	01	05	06	03
	125	03	03	04	02	01
	62.5	00	02	01	00	00
Gentamycin	1000	17	12	12	32	04
Chloramphenicol	1000	09	18	32	20	08

(mm)*= Mean of three replicates
Gent-Gentamycin (10µg/disc),
Chl-Chloramphenicol (30µg/disc),
'-'– No significant result, NT- not tested

Table 3: Antioxidant activity of white tea extracts

Antioxidant activity (Inhibition %)	White tea extracts	Sample concentrations (µg/ml)					IC_{50} (µg/ml)
		100	150	200	250	300	
DPPH	Methanolic	39.10 ±0.01	41.17 ±0.02	46.62 ±0.01	50.98 ±0.01	52.77 ±0.02	250
	Acetone	29.90 ±0.03	32.87 ±0.04	37.45 ±0.02	43.98 ±0.02	50.03 ±0.01	300
ABTS	Methanolic	40.55 ±0.01	43.21 ±0.03	47.68 ±0.01	50.22 ±0.01	52.21 ±0.01	250
	Acetone	38.30 ±0.04	40.71 ±0.01	44.53 ±0.01	47.04 ±0.03	51.13 ±0.01	280
FRAP	Methanolic	32.52 ±0.01	37.06 ±0.02	41.11 ±0.03	46.32 ±0.01	50.11 ±0.03	300
	Acetone	35.85 ±0.01	40.56 ±0.03	46.72 ±0.01	50.98 ±0.02	51.96 ±0.02	280
ORAC	Methanolic	37.85 ±0.02	42.46 ±0.01	46.32 ±0.01	50.62 ±0.01	52.88 ±0.01	250
	Acetone	26.45 ±0.04	28.03 ±0.01	32.88 ±0.03	46.04 ±0.02	50.33 ±0.01	300
SOD	Methanolic	32.25 ±0.02	37.78 ±0.01	40.67 ±0.01	47.45 ±0.03	52.54 ±0.01	300
	Acetone	42.10 ±0.04	46.75 ±0.01	48.86 ±0.03	51.33 ±0.02	53.67 ±0.01	250
Nitric acid scavenging activity	Methanolic	12.10 ±0.02	23.67 ±0.01	35.89 ±0.01	43.34 ±0.01	50.34 ±0.01	300
	Acetone	13.50 ±0.04	26.45 ±0.01	33.18 ±0.03	40.56 ±0.02	51.20 ±0.01	300

All values are expressed as mean ± SD for three determinations
Where, *- No significance **- p<0.01 significance ***- p<0.05 significance

Table 4: Anti-inflammatory activity of white tea extracts

Sample Concentrations (µl)	Anti-inflammatory activity (% Prevention of Lysis)		
	Methanolic extract	Acetone extract	Diclofenac Sodium
100	40.20±0.01	37.50±0.04	48.14±0.001
50	41.80±0.03	35.15±0.02	47.58±0.004
25	38.20±0.04	31.65±0.03	47.08±0.005
12.5	32.15±0.02	29.50±0.01	46.66±0.002
6.3	28.50±0.03	26.75±0.04	45.98±0.001

All values are expressed as mean ± SD for three determinations

Table 5a: Chemical composition of methanolic extract of white tea by GC- MS

Retention Time	Compound Name	Molecular Formula	M.W	Mass %	Area	Area %
2.064	1-(diethylamino)diethylidenmino sulphur pentafluoride	$C_6H_{13}N_2F_{68}$	240	0.2	2.064	0.720
2.366	6-endo-hydroxy-o-exo-methyl-aza-3-oxa bicyclo3,3 amine	$C_7H_{11}O_9N$	157	0.1	2.366	0.825
2.600	1,3-propanediamine,N,N-dimethyl -(CAS)N-N^1-d methane	$C_5H_{14}N_2$	102	0.2	2.600	0.907
3.320	5-hepten-2-amine,N,3-dimethylamine	$C_9H_{19}N$	141	0.5	3.320	1.158
5.698	N-N^{1}diethylfutrescine 1,4 butane diamine ,N,N^1-diethyl amine	$C_8H_{20}N_2$	144	0.5	5.098	1.778
6.864	N,N^1-diethyl-1-methyl-1,3-propane diamine	$C_8H_{20}N_2$	144	0.5	5.098	1.778
12.718	N,N^{1}diethyl-2-methyl-1,3-propane diamine 1,3-propylamine	$C_8H_{20}N_2$	144	0.5	5.098	1.778
13.424	N,N-dimethyl-2-[(2-benzyl) (acetoxy)fhenoxy] ethanoamine	$C_{19}H_{23}C_3N$	313	12.6	6.894	2.405
16.424	isomethepteneoctine	$C_9H_{19}N$	141	42.1	12.424	4.437
19.561	diethylcarbamazine	$C_{10}H_{21}CN_3$	199	20.8	13.424	4.683
20.842	1,3-diethyl-1,3-dazclidine	$C_7H_{16}N_2$	128	15.3	10.424	3.636
21.281	permethylspermine	$C_{16}H_{38}N_4$	286	0.2	19.562	6.824
22.774	C13-1,2-di(1,1-dimethylethyl) cyclopropanecycloethane	$C_{11}H_{22}$	154	0.2	20.812	7.271
24.015	1,3-butanediamine,N,N,N^1,N^1-tetramethyl-(CAS) N,N^1,diamine	$C_8H_{20}N_2$	144	0.2	21.281	7.424
24.210	N,N-dimethyl-2-[(acetoxyphenyl) methylphenoxy]ethylamine	$C_{19}H_{23}C_3N$	313	0.2	22.714	7.924
24.561	ethanol,2-(diethylamino)-(CAS) 2-8.569 diethylaminoethane	$C_6H_{15}ON$	117	0.2	24.019	8.380
27.842	2-propanamine,N-methyl- (CAS) methyl isopropylamine	$C_4H_{11}N$	73	0.2	24.561	8.569
28.211	acetamide,N,N^1[1,4 butane diylbis (ethylamino)]-3,1-propylamine	$C_{22}H_{46}C_2N_4$	398	0.2	27.842	9.713
29.774	azetidine,2,2,3,3-tetramethyl amine	$C_7H_{15}N$	13	0.2	29.774	10.388
32.019	ethanol,2-(diethylamino)-(CAS)2-diethylaminoethane	$C_6H_{15}ON$	117	0.2	32.019	11.171

Table 5b: Chemical composition of acetone extract of white tea by GC- MS

Retention time	Compound name	Molecular Formula	M.W	Mass %	Area	Area %
8.189	Butane1,1-diethoxy-3-methyl	$C_9H_{20}O_2$	160	15.2	8.199	9.436
9.110	Benzeneacetaldehyde	C_8H_8O	120	30.4	9.110	10.484
11.807	Propane 1,1,3-triethoxy	$C_9H_{20}O_3$	176	22.8	11.809	13.588
12.117	1Hindene,1-methylene	$C_{10}H_8$	128	20.3	12.117	13.945
13.305	Sucrose	$C_{12}H_{22}O_{11}$	342	0.2	13.305	15.312
14.466	Teteadecanoic acid	$C_{14}H_{28}O_2$	228	0.2	14.466	16.649
17.864	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.2	17.884	20.582

Figure 1a. Gas Chromatography- Mass Spectrometry profile for methanolic extract of white tea

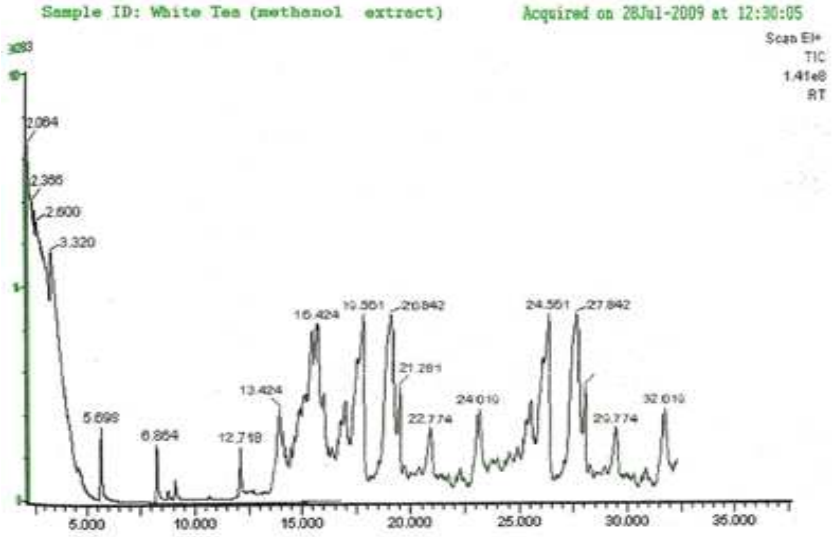
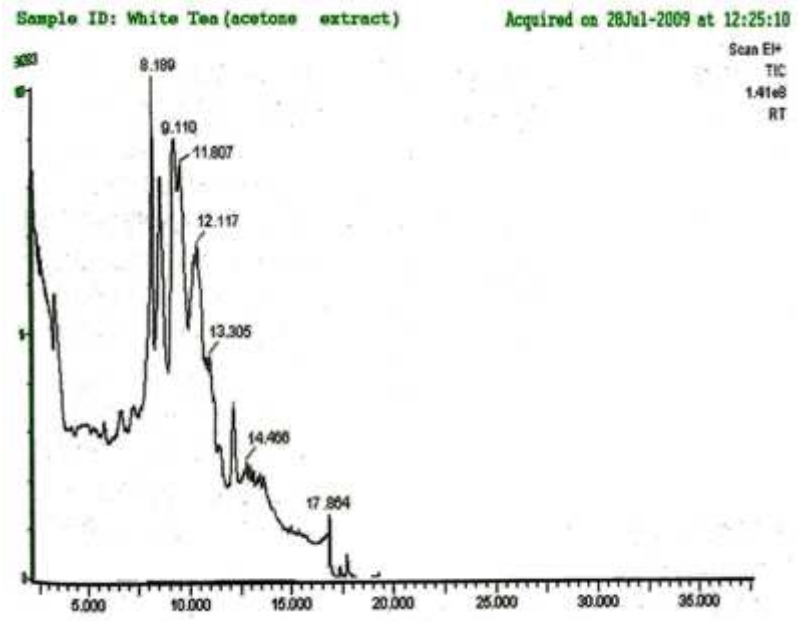


Figure 1b. Gas Chromatography-Mass Spectrometry profile for acetone extract of white tea



Conflict of interest statement

We declare that we have no conflict of interest.

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