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Biochemical and Microbiological Characterization of White Tea

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Abstract: *White tea is just made by drying only without any other normal fermentation process which is adopted for other tea varieties. The biochemical components like flavonoids, total polyphenols, tannins and catcehins are prominent in white tea. Catechin content is higher than tannin content. All those active components are higher in methanolic extracts than their corresponding aqueous extracts. The higher antioxidant activities are manifestations of all those active components. Higher antimicrobial property (inhibition of growth in liquid and solid medium against Gram positive and Gram negative organism) is a beneficial aspect of white tea regarding health concern. Moreover lowering of in vitro blood glucose level and inhibition of digestive enzymes (α amylase, β-D Glucosidase, lipase) may attribute to the biomedical application of white tea.*

Key words*: White tea, antimicrobial, antioxidant, digestive enzyme.*

I. Introduction

White tea is an unfermented tea produced from young shoots of *Camellia sinensis* (Alcazar et al., 2007).It is non-fermented tea and usually protected from sunlight to avoid polyphenol degradation. It includes withering, piling, rolling and drying. In white tea epigallocatechin gallate (EGCG) is the main active compound made from *Camellia sinensis*, followed by epigallocatechin (EGC; 3.25%), gallocatechin (GC; 1.28%), and catechin (C; 0.71%) which were also found in the extract (Teixeira et al.,2012). White tea is a rare variety, its particular post-harvest processing method raises small silvery hairs on the dried buds. White tea contains a higher proportion of the buds which are covered with fine "silvery" hairs that impart a light white color to the tea (Karori et al., 2007). It is made by partial steaming and air-drying in natural sunlight. Karori et al., 2007 stated that green and white teas' had significantly ($p< 0.05$) higher antioxidant activity compared to black tea. White tea is not rolled or crushed but it is steamed rapidly and air-dried to preserve most of the polyphenols. Sohle et al., 2009 had demonstrated that components of green tea are somewhat comparable to those of white tea. They had proposed that white Tea extract induces lipolytic activity and inhibits adipogenesis in human subcutaneous (pre)-adipocytes. White, green and black teas differ in their sensorial properties and have markedly different chemical compositions (Wang & Ho, 2009). White tea is a seasonal crop (spring) with special sensory and health benefits. White tea is lower in caffeine than green tea (Hilal and Engelhardt, 2007). According to them white tea has a higher anti-mutagenicity action compared to green tea. They had distinguished the biochemical characters from that of green, oolong and black tea. Unachukwu et al., 2010 had made in-depth chemical analysis of white tea types and conducted tests to quantify polyphenols and antioxidant potential of 8 commercially available white teas, and compared them with green tea. The manufacture, chemistry and health effects of White tea is elaborately stated by Jiang, 2008. Zhao et al., 2011 had identified, quantified and characterized the principal components of white tea.

II. Material AND Methods

- ***Tea samples:***

White tea samples were acquired from market (Mahabodhi Tea House, Rashbehari Avenue outlet, Kolkata). 500 mg tea leaves were taken for aqueous and methanolic extract preparation (in 10 ml volume).

White Tea manufacturing process: Fresh tea leaves→ Withering→ Drying→ White tea

- **Measurement of tea colour:** The tea colour was measured according to Goodner and Wampler, 2008—measured hue at 460nm wavelength.(Fig 1)
- **pH determination:** pH of different solutions of tea were measured according to Saadeh et al, 2009. (Fig 2)
- **Moisture and ash content determination:** Moisture content was measured by using hot air oven at temperature of 105°C by following the Anon., 2000 and ash content was measured according to Srilakshmi, 2003 by using muffle furnace at 500–600°C for 5–6 hrs. (Fig 3)
- **Thin layer chromatography:** It was done according to Amarowicz et al., 2005 using silica as stationary phase and Vanillin-HCl as spraying agent (Fig 4)

- **Spectrophotometric scan within UV range (200-400nm):** The scan was done according to Atomssa and Gholap, 2015(Fig 5)
- **Antioxidant activity:** The antioxidant activity was measured according to Ribeiro et al., 2002(Fig 6)
- **Antimicrobial activity:** The inhibition of growth in liquid culture and solid medium were done according to Saha and Shyam Choudhury, 2016. (Fig 7, 8, 9,10)
- **Antidiabetic action:** Antidiabetic action of tea extracts determined with Glucose kit-(Satter et al., 2012)(Fig 11)
- **Inhibition of digestive enzymes:** The inhibition of digestive enzymes was done according to Li et al., 2015. (Fig 12)
- **Assay of total polyphenols:** It was determined (Table 1) according to Anesini et al., 2008 with 1:10 Folin Ciocalteu reagent, and 4 ml of 7.5% Na_2CO_3
- **Assay Tannins:** According to modified method of Broadhurst et al., 1978 tannins was assayed (Table 1) by mixing with 3 ml of vanillin solution, 1.5 ml of concentrated HCl and incubated then OD was taken at 500 nm.
- **Assay of Catechins:** Catechin was determined according to Wallace, 2010 with 1:10 Folin-Ciocalteu reagent, and 4 ml of 7.5% Na_2CO_3 and the absorbance was taken at 765 nm.
- **Assay of total flavonoids:** The flavonoid assay was done according to Zhishen et al., 1999 by mixing the tea extracts with 75 µl of 5% Na_2CO_3, 150 µl of 10% of $AlCl_3$, 750 µl of NaOH and 2500 µl of water, and the O.D was measured at 510nm (Table 1).
- **Assay of caffeine:** The caffeine content was measured (Table 1) according to Adnan et al., 2013**,** by addition of water, 0.01 mol/l HCl, 2 ml basic lead acetate solution and O.D was measured at 274nm.

III. Results AND Discussion:

- **Measurement of colour**: The colour of white tea is more or less pale (O.D at 460nm is .053±0.001 /mg fresh wt.) compared to black tea (O.D at 460nm is0 .240±0.01 /mg fresh wt.-Saha and Shyam Choudhury, 2016)(Fig 1). The pale yellow color of brewed white tea was reported by Karori et al., 2007.
- **pH determination**: The pH of the aqueous and methanolic extracts were almost same (methanolic-4.75, aqoueous-4.81). (Fig 2). pH of white tea extracts were discussed by Peiro et al., 2014.
- **Moisture content and ash content**: The moisture content of the white tea is 16±0.01% and ash content is 6±0.01 %.(Fig 3). Ekayanti et al., 2017 had demonstrated the same ash and moisture content (Physico-chemical parameters) of white tea.
- **Thin layer chromatography**: The thin layer chromatography has shown the intense band for catechin (specially epigallocatechin gallate) in methanolic extract than that of aqueous extracts of white tea. (Fig 4). Ekayanti et al., 2017 had also demonstrated the presence of spots in alcoholic extracts of white tea establishing the presence of catechins.
- **Spectrophotometric scan within UV range (200-400nm)—**the spectrophotometric scan within 200-400nnm had shown the prominent peaks of methanolic extracts showing the higher active catechin content. (Fig 5). Atomssa and Gholap, 2015 showed that the catechins have absorption within 246–316 nm.
- **Antioxidant activity**: The antioxidant activity of methanolic extracts (78.9±0.01%) of white is higher than that of aqueous extract (65±0.01%) (Fig 6). Almajano et al., 2008 had demonstrated antioxidant activity of white tea.
- **Antimicrobial activity**: The inhibition of liquid growth of *E.coli* of Methanolic white tea extract is 32±0.01% and of aqueous extract is 4±0.01%. Against *B.subtilis* for methanolic extract is 27±0.01% and of aqueous extract is 10±0.01% (Fig 10). The zone of inhibition of methanolic extract against *E.coli* and *B.subtilis* are 2.2 and 1.5cm respectively, and for aqueous extracts 1.1 and 0.9 cm respectively (Fig 7,8,9). Holloway et al., 2011 had demonstrated the antimicrobial activity of white tea against *Staphylococcus aureus.*
- **Antidiabetic action:** Methanolic extract has power to lower the blood glucose level in vitro 20±0.01% and aqueous extract lower 3±0.01%. (Fig 11). Cooper, 2015 had shown the lowering of blood sugar by white tea.
- **Inhibition of digestive enzymes**- α amylase is inhibited 51±0.01% by white tea methanolic extract and 13±0.01% with aqueous extract. β-D Glucosidase is inhibited 42±0.01% by white tea methanolic extract and 14±0.01% with aqueous extract and Lipase is inhibited 40±0.01% by white tea methanolic extract and 10±0.01% with aqueous extract. (Fig 12). Gondoain et al., 2010 had demonstrated the inhibition of lipase activity in vitro with white tea.
- **Total polyphenol content (%) content**: For methanolic extract 35±0.01% and for aqueous extract 29±0.01%. (Table 1). The high polyphenol content responsible for antiseptic and antioxidant properties of white tea were described by Ekayanti et al., 2017.

- **Catechin content**: Total catechins 19±0.01% in methanolic extracts and 13±.01% in aqueous extracts of white tea were found. Ekayanti et al., 2017 had demonstrated almost same level of catechins (18.94±0.01%) in white tea samples. Kumar et al., 2011 have stated that among all the tea types, white and green tea has the highest concentration of catechins. Various types of bioactive compounds found in white tea leaves such as polyphenols, caffeine, and catechins especially epigallocatechin gallate (EGCG), were described by Unachikwu, 2010.
- **Tannin content**: Tannin content 11 ±0.001 (%w/w) and 9±0.001 (%w/w) in methanolic and aqueous extracts of white tea respectively.(Table1).
- **Total Flavonoid content**: Total flavonoid content in gm/100gm is 5±0.001 in methanolic extract and 1±0.001 in aqueous extract.(Table 1). Azman et al., 2014 had demonstrated the high quality flavonoids and its radical scavenging activity.
- **Caffeine content**: 4.8±0.01gm/100gm and 3.9±0.01gm/100gm are the caffeine content in methanolic and aqueous extracts of white tea. (Table 1). The similar amount of Caffeine content (average 4.85gm/100gm) was described by Hilal and Engelhardt, 2007.

IV. Conclusion

The presence (in higher amount) of active biochemical components like flavonoids, total polyphenols, catechins render higher antioxidant and antimicrobial activities of White tea which makes it superior. Ekayanti et al., 2017 had shown pharmacognostic and phytochemical characterization of white tea leaf with respect to flavonoid, catechins bioactive component analysis. Lowering of blood glucose level and inhibition of digestive enzyme adds up biomedical importance of white tea. Moreover inhibition of such enzymes makes white tea as anti-obesity factor. The bioactive action of white tea flavonoids were described by Azman et al., 2014.

Fig 1. Colour of solid and aqueous extract of white tea

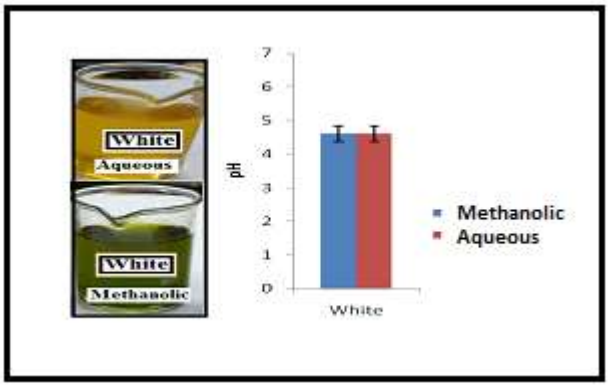

Fig 2. The pH of methanolic and aqueous extract of white tea

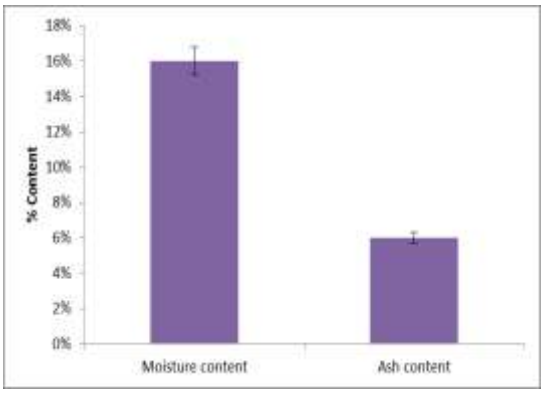

Fig 3. Moisture content and ash content of white tea

Fig 4. Thin layer chromatography of methanolic and aqueous extracts of white tea.

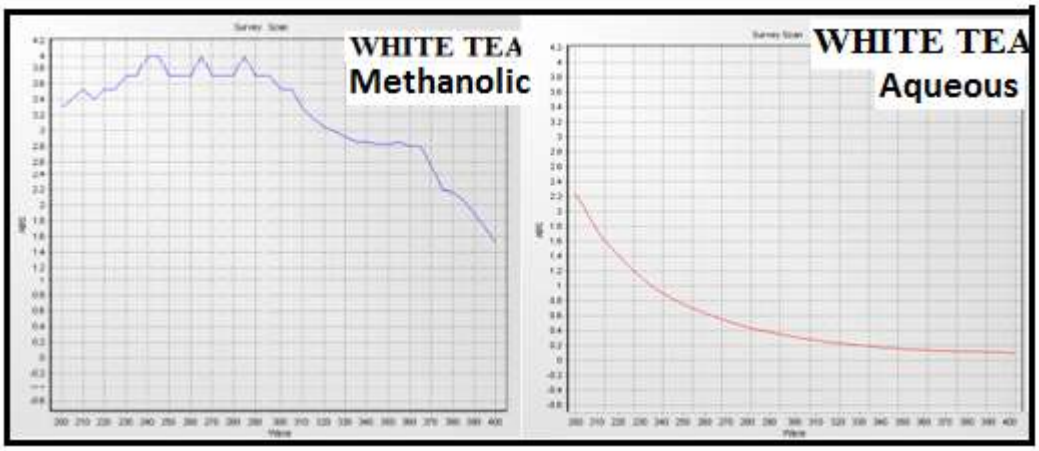

Fig 5. Spectrophotometric scan within 200-400nm of methanolic and aqueous extracts of white tea.

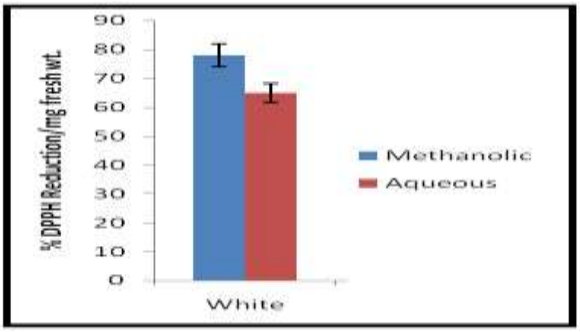

Fig 6. Antioxidant activities of methanolic and aqueous extracts of white tea.

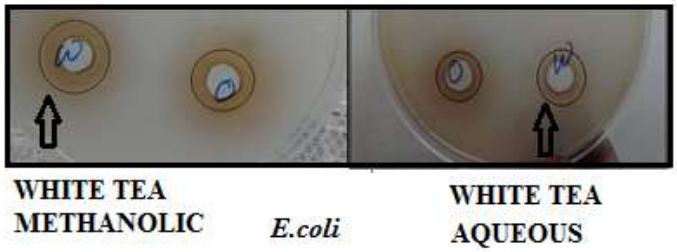

Fig 7. Antimicrobial activity measurement in Muller-Hinton medium against *E.coli*

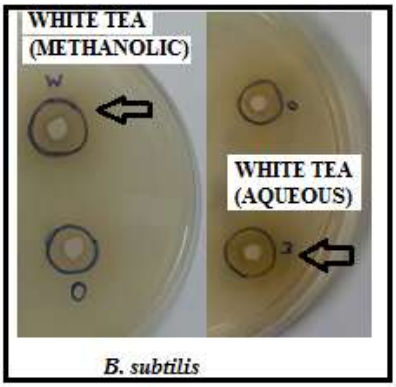

Fig 8. Antimicrobial activity measurement in Muller-Hinton medium against *B.subtilis*

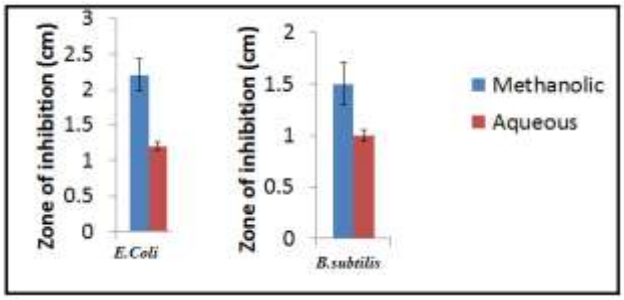

Fig 9. Measurement of Zone of inhibition in Muller Hinton agar medium

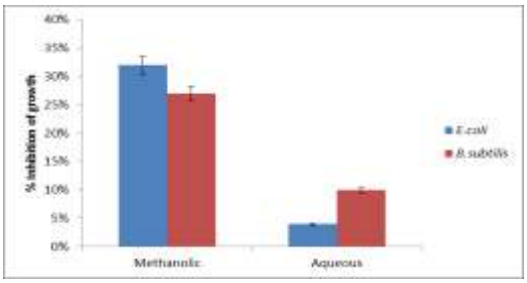

Fig10. Inhibition of growth in liquid nutrient broth culture.

Table 1: Assessment of Biochemical components of White tea:

Biochemical Components	White tea (Methanolic)	White tea (Aqueous)
Total Polyphenols (%)	35±.01	29±.01
Catechins (%)	19±.01	13±.01
Total Flavonoids (gm/100gm)	5±.001	1±.001
Caffeine (gm/100gm)	4.8±.01	3.9±.01
Tannins (%w/w)	11±.001	9±.001

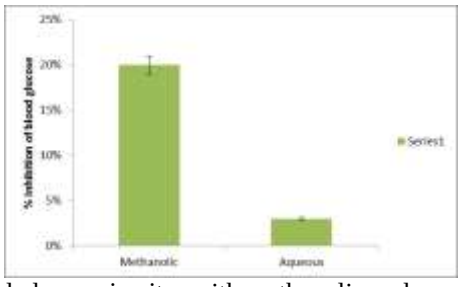

Fig 11. Inhibition of blood glucose *in vitro* with methanolic and aqueous extracts of white tea.

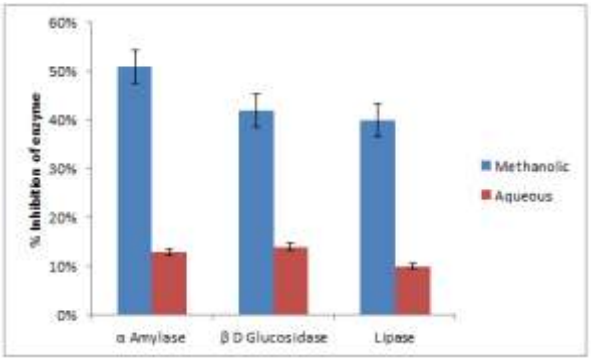

Fig 12. Inhibition of digestive enzymes with methanolic and aqueous extracts of white tea.

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