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Phytochemical Screening, *Invitro* Antioxidant, Anti-Diabetic, Anti-Inflammatory Activity Of Green Tea And Black Tea Leaves Extract

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Abstract

Tea is not only a popular drink but also a drink with refreshing and functional properties. Thus, the green and black tea leaves were collected and used for preparing green and orthodox black tea to study antioxidant activity, phytochemicals profile such as Antioxidant activities, Anti-diabetic studies, and Anti-inflammatory study along with Anti-microbial activity. The contemporary scientific community has presently recognized flavonoids to be a unique class of therapeutic molecules due to their diverse therapeutic properties. Of these, rutin, also known as vitamin P or rutoside, has been explored for a number of pharmacological effects. Tea leaves, apples, and many more possess rutin as one of the active constituents. Today, rutin has been observed for its nutraceutical effect. The present study highlights the anti-diabetic, anti-inflammatory, anti-microbial and antioxidant effects of rutin. The rutin present in the green tea leaves and black tea leaves are herein mainly focussed and compared.

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Keywords: Green and Black tea leaves, Anti-inflammatory, Anti-diabetic, Antimicrobial, Antioxidant,

INTRODUCTION

Tea plant (*Camellia sinensis L.*) is such a source to provide us a tea brew that's so refreshing and further, it is a popular drink in the world. It has been well defined as an infusion aqueous and as well as hot of dried leaves. That is to say, it is all an extract of leaves, leaf nodes, and further as of inter-nodes of the plant (*Camellia sinensis L.*) (1). They have not only the stimulating effect but also therapeutic properties because of polyphenolic compounds present in them. In this study, green tea was virtually, when compared to the black teas, were found to have rather higher concentrations of phenolic compounds and the major antioxidant activities than black tea. It seems drinking black tea has indeed benefits equal to those of drinking green tea in terms of their antioxidant capacities and such is the case, because theaflavins that are available in black tea leaves possess at least the same antioxidant potency as catechins available in green tea (2).

Fig 1: Green tea leaf

In this study, it is introduced as to a conclusion such as that both green and black tea leaves possess a marked anti-inflammatory effect against all such denaturation of protein, *in- vitro* and further, green tea is found to be more active than black tea, most plausibly because of the higher flavonoid contents of green tea (5). It seems apparently that green tea has more health benefits than an equal volume of black tea when deemed in terms of antioxidant capacity and such a statement can be explained by the fact that each tea is different in terms of composition as well as concentration of antioxidant compounds(6). Tea is one of the most-widely consumed beverages in the world with a number of different beneficial health effects, mainly ascribed to the polyphenolic content of the tea catechins (7). Further, rutin study is intensified and its presence in both green tea leaves and black tea leaves are studied comparatively.

Flavonoids and polyphenolic compounds are one of the important classes of plant derived chemicals that do contain indeed benzopyrone moiety. About 4000 types of flavonoids have been reported or announced authoritatively to be present in plants (3). Rutin is a flavonol, and it is found in an abundant measure in passionflower, buckwheat, tea, and apple. Without a doubt, it is a cardinal nutritional component of food; Rutin is also called as rutoside. Rather the name 'rutin' comes well from the plant Ruta graveolens, which also contains rutin. Chemically rutin is nothing but a glycoside that comprises of flavonolic aglycone quercetin along with disaccharide rutinose. No doubt, rutin has performed many pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities. Green tea is deemed to be anti-inflammatory, antioxidative, antimutagenic, and anticarcinogenic, and can very much prevent even the cardiac disorders (4). Many of these beneficial effects of green tea are due to its catechin, that is to say, particularly (−)-epigallocatechin-3-gallate (EGCG) content assuredly. To find out as to how the beneficial effects of black tea due to its contents, in this study, a comparative study is made and introduced. Green tea leaves and black tea leaves are excellent α-glucosidase inhibitors, and the findings suggest that green tea and black tea can potentially be used to control postprandial hyperglycemia (5). But which teas contain more α-glucosidase inhibitors, and such a question will arise and for the answering of such a question too this comparison study is explored and presented here. Through cellular, animal, and also through human experiments, it has already been shown that the green tea leaf and its major component such as epigallocatechin-3-gallate (EGCG) have anti-inflammatory effects (6). But whether the black tea leaf has the same anti-inflammatory property or efficacy, and such a question will arise and for the answering of such a question too, this comparison study is explored and presented here. Green tea is, of course, a non-fermented tea. Again and again it has come to be known and valued for its medicinal properties (7). But whether black tea too has medicinal properties and such questions will arise and hence, towards the clarifications as towards such questions, this comparison has been explored successfully and herein the same is introduced. In the present study phytochemical screening, *invitro* antioxidant, anti-diabetic, anti-inflammatory, activity of green tea and black tea leaves extracts.

MATERIALS AND METHODS

Green Tea Leaves

The fresh tea leaves were collected from the hills of Nelyampathy tea estate located in Palakkad, Kerala. The leaf buds used for green tea sampling were properly cut from the tea plants, washed and dried well in shadow. Once the leaves were dry, they were grinded in mortar and pestle to a fine powder.

Fig: 2. Green tea Leaves

The solvents used were Aqueous, Ethanol and N – Hexane. Dissolved 2g of powdered sample in 20ml of each solvents, shaken overnight. The next day filtered the final supernatant (the sample or the extract) which was used for further phytochemical studies.

Black Tea Leaves

The solvents used were Aqueous, Ethanol and N – Hexane. Dissolved 2g of powdered sample in 20ml of each solvents, shaken overnight. The next day filtered the final supernatant (the sample or the extract) which was used for further phytochemical studies.

Fig: 3. Black Tea Leaves

Fig: 4. Extract of Green Tea Leaves

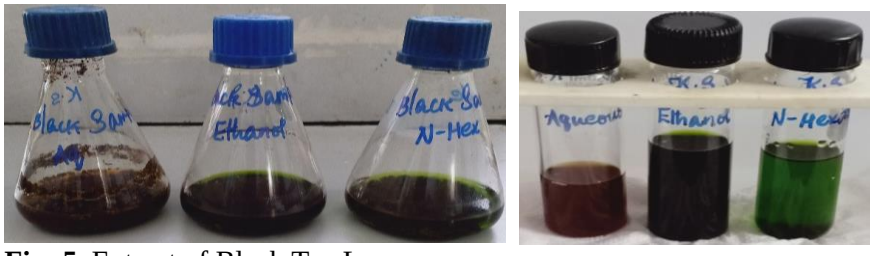

Fig: 5. Extract of Black Tea Leaves

Fine grinded tea leaves were soaked in solvents and the extracts were obtained after overnight soaking**.**

Phytochemical screening and Antioxidant activities

DPPH assay:

The antioxidant property of rutin compound was analyzed by DPPH assay. 0.1ml of 0.1N DPPH was added to 0.5ml of sample and incubated for 5min. Then 0.4ml of 50mM Tris HCL was added incubated for 30 minutes at room temperature and checked OD at 517nm.

Flavanoids:
To 1ml of extract added 0.1ml of AlCl3 (10%), 0.1ml of potassium sodium tartarate (1M), 2.8ml of distilled water and incubated at room temperature for 30 minutes. Checked the OD at 415nm.

Total Phenol:
To 1ml of extract added 0.2ml of Folin's phenol reagent, 1 ml of 20% Sodium Carbonate, incubated at 45℃ for 45 minutes in water bath and checked OD at 765nm.

Phosphomolybdenum method:
To 0.5ml of sample, added 0.5ml of reaction mixture, mixed well and incubated at 50℃ for 90 minutes. Checked OD at 695nm. We get the following results:

Superoxide dismutase (SOD):
To 1 ml of sample added 1 ml of SOD reaction mixture I, incubated at 30℃ for 5 minutes, added 80µl of 50mM riboflavin, mixed well and exposed under 200v fluorescent light for 3 minutes. Then added 1ml of reaction mixture 2, mixed well and checked OD at 543nm.

Anti-diabetic studies:
α – Amylase enzyme activity:
To 1 ml of sample, added 0.1% starch solution in 16mM Sodium acetate buffer, 0.2ml of α – amylase, 96mM sodium potassium tartarate and 3,5 dinitro salicylic acid, mixed well and incubated in alkaline condition at 25℃ for 5 – 10 minutes. The reaction was detected at 540nm.

α – Glucosidase enzyme activity:
To 1 ml of 2% starch substrate, added 1ml of sample mixed well and added 1ml of 0.2M tris buffer (pH 8). Mixed well and incubated at 37℃ for 15 – 30 minutes. Then added 0.2ml of α – Glucosidase, incubated at 35℃ for 40 – 45 minutes, added 2 ml of 6N HCl to terminate the reaction and measured the OD at 540nm.
Calculation (50% of inhibition)
% of inhibition = (control / OD of sample) / C X 10

Anti-inflammatory study: Antiproteinase action (Trypsin):
Table 1: Phytochemical Test—Black Tea

Sample	Aqueous	Ethanol	N-Hexane
Alkaloids	+	-	-
Terpenoids	-	-	-
Phenols	+	+	-
Sugars	+	-	-
Saponins	+	-	-
Flavanoids	+	-	-
Quinines	+	-	-
Protein	-	+	-
Steroids	+	-	-

Mixed 0.06mg of trypsin, 1ml of 20mM Tris Hcl buffer and 1ml test sample, the mixture was incubated at 37℃ for 5 minutes and 1ml of 0.8% casein was added. The mixture was incubated for 20 minutes, then 2ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of supernatant was read at 210nm against buffer as blank.
% of inhibition = (control – sample) / control X 100

RESULTS AND DISCUSSION

Phytochemicals screening
As according to the phytochemical Test for Green Tea, there is no positive result at all for N-hexane solvent whereas for Black tea, there is still a possible one positive result. With Aqueous solvent, for Black tea,

Terpenoids alone shows its absence whereas with Green tea, with aqueous solvent, both Terpenoids and Protein show their absence.

Table 2: Phytochemical Test—Green Tea

Sample	Aqueous	Ethanol	N-Hexane
Alkaloids	+	+	-
Terpenoids	-	-	-
Phenols	+	+	-
Sugars	+	+	-
Saponins	+	+	-
Flavanoids	+	-	-
Quinines	+	-	-
Protein	+	+	+
Steroids	+	+	-

Phytochemical activities:

DPPH assay:

Thus, when we analyse the antioxidant property of rutin compound, as per DPPH assay, we get for Green tea leaves as: Aqueous – 1.138, Ethanol – 0.869, and N – Hexane – 0.248 whereas for Black tea leaves, we get the same three units or measures as differing as: Aqueous – 0.225, Ethanol – 0.301, and N – Hexane – 0.305. The solvents used were Aqueous, Ethanol and N – Hexane. For Aqueous and Ethanol solvents, black tea leaves show less antioxidant activities rather than green tea leaves. The green tea shows a higher antioxidant levels than black tea resultantly (8).

Flavanoids:

Thus, when we analyse for the Flavanoids of rutin compound, we get for Green tea leaves as: Aqueous – 0.689, Ethanol – 1.634, and N – Hexane – 0.707 whereas for Black tea leaves, we get the same three units or measures as differing as: Aqueous – 2.091, Ethanol – 2.561, and N – Hexane – 0.261. The solvents used were Aqueous, Ethanol and N – Hexane. For Aqueous and Ethanol solvents, black tea leaves show more Flavanoids rather than green tea leaves. Tea flavonols or flavonoids are potent antioxidants and make up rather 2–3% of the water-soluble solids from tea leaves (9). A total number of 203 flavonoids were, of course, identified apparently during black tea processing.

Total Phenol:

Thus, when we analyse for the Phenol content of rutin compound, we get for Green tea leaves as: Aqueous – 1.500, Ethanol – 1.764 and N – Hexane – 1.194 whereas for Black tea leaves, we get the same three units or measures as differing as: Aqueous – 0.201, Ethanol – 0.046 and N – Hexane – 0.008. The solvents used were Aqueous, Ethanol and N – Hexane. For Aqueous, Ethanol, and N – Hexane solvents, Green tea leaves show more phenol rather than Black tea leaves.

Phosphomolybdenum method:

Thus, when we analyse for the total antioxidant property of rutin compound by means of Phosphomolybdenum method, we get for Green tea leaves as: Aqueous – 0.570, Ethanol – 0.194, and N- Hexane – 0.028 whereas for Black tea leaves, we get the same three units or measures as differing as: Aqueous – 0.264, Ethanol – 0.229 and N- Hexane – 0.014. The solvents used were Aqueous, Ethanol and N – Hexane. For Aqueous and N – Hexane solvents, Green tea leaves show more total antioxidant property rather than Black tea leaves. Further, herein it is to be pointed out that the basic principle to assess the antioxidant capacity through phosphomolybdenum assay includes a reduction such as the reduction of Mo (VI) to Mo (V) by sheer means of the plant extract having antioxidant compounds.

Superoxide dismutase (SOD):

Thus, when we analyse for the SOD of rutin compound, we get for Green tea leaves as: Aqueous – 2.202, Ethanol – 2.555, and N – Hexane – 0.201 whereas for Black tea leaves, we get the same three units or measures as differing as: Aqueous – 1.414, Ethanol – 1.520 and N – Hexane – 0.261. The solvents used were Aqueous,

Ethanol and N – Hexane. For Aqueous and Ethanol solvents, Green tea leaves show more SOD rather than Black tea leaves. Further, the effect of tea catechins as indeed as towards the free radicals is, of course, multidirectional and incorporates an enhancement such as the enhancement of endogenic antioxidant enzymes production superoxide dismutase and glutathione] and a protection as well as a regeneration such as the protection and regeneration of antioxidant compounds (vitamin C or E).

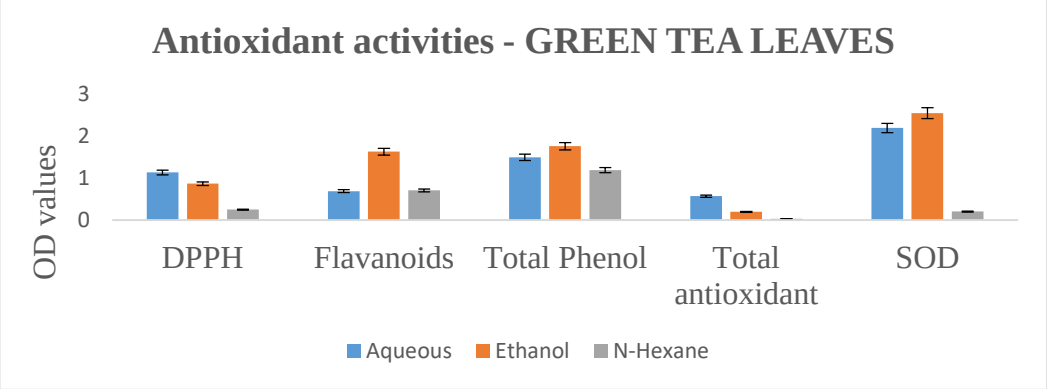

Fig: 6. Antioxidant activities of Green Tea Leaves

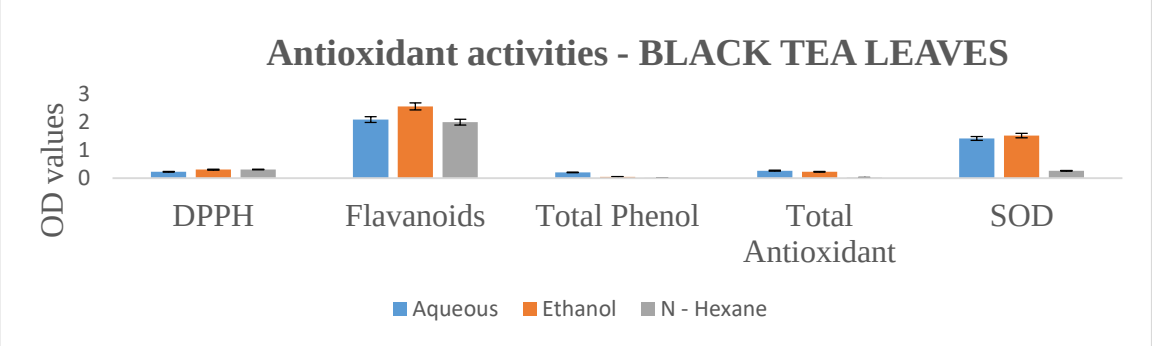

Fig: 7. Antioxidant activities of Black Tea Leaves

Anti-diabetic studies:

α – Amylase enzyme activity:

Thus, while we are unto be finding the Anti-diabetic property, we inherently analyse the enzyme activity of the rutin compound and thus, when we analyse for the enzyme activity of the rutin compound, we get for Green tea leaves as: OD – 540nm, C – 0.000 and E – 2.466 whereas for Black tea leaves, we get the same three units or measures as differing as: OD – 540nm, C – 0.000 and E – 0.674. The solvents used were Aqueous, Ethanol and N – Hexane. We can thus blindly and resultantly can come to a conclusion that the anti-diabetic activity or enzyme activity of the Green tea leaves is far exceeding than the Black tea leaves.

α – Glucosidase enzyme activity:

Thus, while we are unto be finding the Anti-diabetic property, we inherently analyse the α – Glucosidase enzyme activity of the rutin compound and thus, when we analyse for the α – Glucosidase enzyme activity of the rutin compound, we get for Green tea leaves as: OD – 540nm, C – 0.000, E – 0.972 whereas for Black tea leaves, we get the same three units or measures as differing as: OD – 540nm, C – 0.000 and E – 0.427. The solvents used were Aqueous, Ethanol and N – Hexane. We can thus blindly and resultantly can come to a conclusion that the anti-diabetic activity or α – Glucosidase enzyme activity of the Green tea leaves is far exceeding than the Black tea leaves.

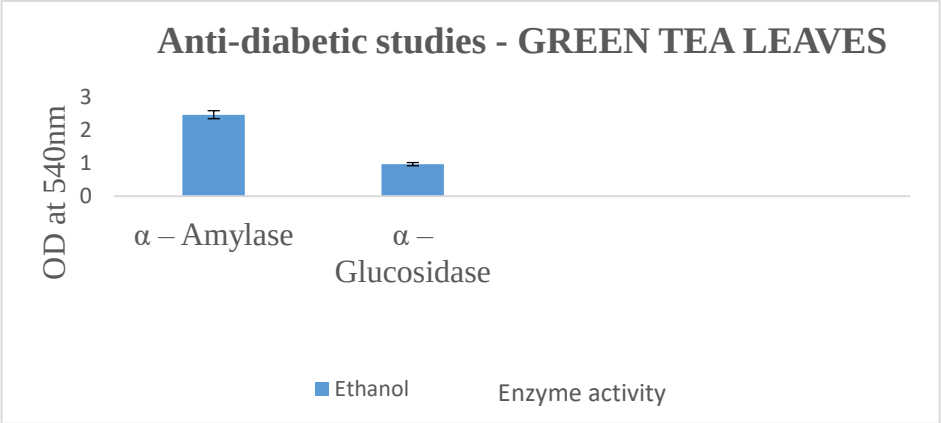

Fig: 8. Anti-diabetic activities of Green Tea Leaves

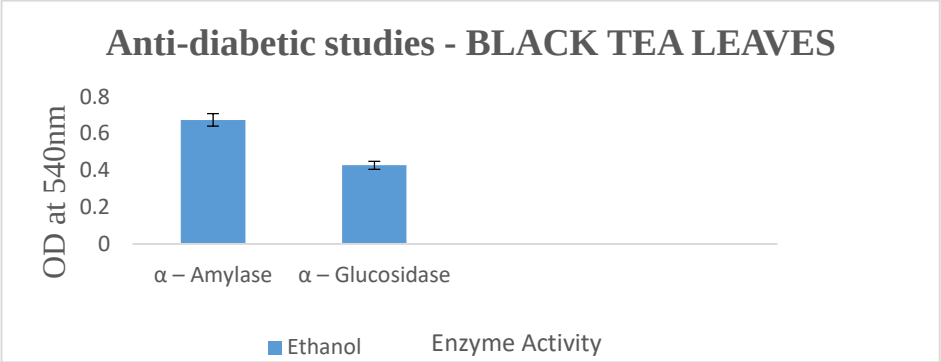

Fig: 9. Anti-diabetic activities of Black Tea Leaves

Anti-inflammatory study: Antiproteinase action (Trypsin):
Thus, while we are unto be finding the Anti- inflammatory property or activity, we inherently analyse the Antiproteinase action (Trypsin): of the rutin compound and thus, when we analyse for the Antiproteinase action (Trypsin): of the rutin compound, we get for Green tea leaves as: Trypsin – 210nm, C – 0.000 and E – 0.326 whereas for Black tea leaves, we get the same three units or measures as differing as: Trypsin – 210nm, C – 0.000 and E – 0.133 .The solvents used were Aqueous, Ethanol and N – Hexane. We can thus blindly and resultantly can come to a conclusion that the anti-inflammatory activity or Antiproteinase action (Trypsin) of the Green tea leaves is far exceeding than the Black tea leaves. Green tea has been detected as having beneficial effects against a variety of ailments or diseases like cancer, obesity, diabetes, cardiovascular disease, and further against neurodegenerative diseases (10).

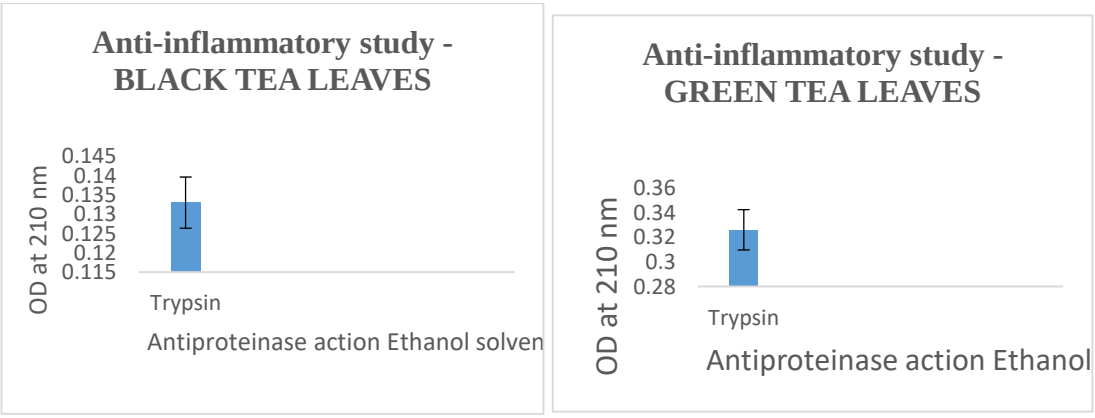

Fig: 10. Anti-inflammatory activities of Green Tea Leaves and Black Tea Leaves

CONCLUSION

As evident from aforesaid facts, Rutin is a phytochemical with multiple pharmacological activities and has a wide array of biological activities. Hence, Rutin can be regarded as a 'vital phytochemical' which is needed to

be studied extensively to establish effective safety profile in human to get therapeutic benefits. Rutin both in Green tea leaves as well as in Black tea leaves provide an ample proof as to its enormous medicinal characters or therapeutic characteristics. The rutin in black tea leaves contains health benefits due to the various secondary metabolite's activities to overcome diabetes, lung cancer, prostate cancer, breast cancer, anti-ulcer, and respiratory disorders. It has been proved that the polysaccharides available both in green tea leaves as well as in black tea leaves are safe and non-toxic so that these can be used in medicine rather.

CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

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