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GC-MS Profiling and *in silico* Polypharmacology Establish Antioxidant Rich Purple Tea as a Major Functional Beverage against Various Lifestyle Diseases

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Abstract: Purple tea (PT) is a high-antioxidant tea variety that is rich in anthocyanin. The rich purple color and antioxidant nature of the drink have provoked several studies on the plant. Some studies highlight the therapeutic potential of the plant. In this paper, we performed phytochemical and polypharmacological studies on samples collected from Barnesbeg Tea Garden, Darjeeling, India. We have employed *in silico* reverse pharmacology and a target-fishing approach to comprehend the full potential of PT in various disease management. Twenty-nine phytocompounds were obtained in the Gas Chromatography-Mass Spectrometry analysis of PT leaves. Antioxidant property assessment of PT through DPPH radical scavenging activity and FRAP assay showed much higher activity than standards, ascorbic acid, and BHT, respectively. The total phenol and flavonoid were quantified as 19.36 ± 0.44 mg GAE/g and 571.801 ± 1.44 mg QE/g of extract, respectively. It was found to target pathways such as the ErbB signaling pathway, PPAR signaling pathway, Insulin signaling pathway, Ras signaling pathway, Natural killer cell-mediated cytotoxicity, Neurotrophin signaling pathway, etc. This is the first study on target fishing and network pharmacology of PT. Thus, the study exhibited PT's role and probable mechanism of action against type-2 Diabetes, Hyperlipidemia, Coronary Heart Disease, Cancer-related impediments, and other lifestyle disorders.

Keywords: purple tea; *Camellia sinensis*; lifestyle ailments; GC-MS; network pharmacology; functional annotation.

1. Introduction

A new anthocyanin-rich tea called Purple tea (PT) (*Camellia sinensis* var. kitamura) was introduced in 2009. It has high antioxidant properties, and the compounds present in it act as an anti-obesity agent and suppress fat absorption [1,2]. The variety is even reported to be highly resistant to tea's major diseases and pests [3]. The wild PT plant was first discovered in the gardens of Assam, India, originating from the *Camellia sinensis* var. assamica plant [4]. However, the credit for the development and commercialization of this variety goes to Kenya. Since 2014, experimentation to release and develop planting materials of PT has been initiated by Darjeeling Tea Research & Development Centre, Tea Board of India, Kurseong, India [4]. Though few of the health benefits of PT have been explored, the full pharmacological potential of the plant (especially PT from India) is largely elusive.

Li *et al*. [5] compared the chemical components of PT with that of Yunkang green tea through a metabolomics approach based on ultra-high performance liquid chromatography (UHPLC)-Orbitrap-tandem mass spectrometry (MS/MS). This gave an idea regarding the probable metabolic pathways of the plant. However, the overall effect of PT on different metabolic pathways of humans is still not explored. Multiple phytocompounds present in plants interact with multiple proteins and pathways of our body's complex system and may aid in disease management [6]. Network pharmacology is an approach that aids in studying the interaction of these phytochemicals and proteins. It creates a scope for multi-faceted and multicomponent therapy in the field of disease management and drug discovery [7].

Moreover, the increasing competition and unhealthy diets in the present society have become major stress contributors to people's life. According to WHO, the world is facing more than 70% of death due to non-communicable lifestyle ailments (https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases) such as cardiovascular problems, type 2 diabetes, anxiety, obesity, etc. In this context, PT can contribute as a stress-relieving beverage.

Therefore, we attempted to explore the unknown potentials of PT from Darjeeling Hills through phytochemical analysis and network pharmacology. Network pharmacology will help to understand the mode of action of PT in known fields. The study will also help identify phytoconstituents of PT, other than anthocyanin, that may help manage different stress-related diseases.

2. Materials and Methods

The study comprises two phases, i.e., phytochemical analysis and *in silico* polypharmacological investigation.

2.1. Phytochemical studies.

2.1.1. Chemicals and reagents.

Chemicals and solvents used for the present study were of analytical grade and purchased either from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Merck (Mumbai, India), or Sigma-Aldrich (USA).

Chemicals used for DPPH assay:

2,2- diphenyl-1-picrylhydrazyl (DPPH) (1mM), methanol, ascorbic acid (standard).

Chemicals used for Ferric reducing antioxidant power assay:

Sodium phosphate buffer (0.2M, pH 6.6), $K_2Fe(CN)_6$ 1(% w/v), TCA (10%), $FeCl_3$ (0.1% w/v), Butylated hydroxytoluene (standard).

Chemicals used for total phenol estimation:

10% Foling reagent diluted (9:1) using double distilled water, Gallic acid (standard).

Chemicals used for total flavonoid estimation:

5% $NaNO_2$, deionized water, $AlCl_3$ (10%), 1mM NaOH, Quercetin (standard).

2.1.2. Instruments.

Magnetic stirrer (Spinot Digital Model MC02, Tarsons make), UV- Visible spectrophotometer (Model- UV-1800, Shimadzu Corporation, Kyoto, Japan), GC- MS QP2010 Plus (Shimadzu Corporation, Kyoto Japan), Centrifuge (Remi C-24 plus), Micropipettes (Microlit) were used.

2.1.3. Sample collection and extract preparation.

The PT cultivated and manufactured in Barnesbeg Tea Garden, Darjeeling, West Bengal, India (27.1030° N, 88.2648° E) was used in this study. The purple-colored foliage (Figure S1: A, B) of PT plants is processed and marketed. This processed tea (Figure S1: B) was purchased from Margaret's Hope, located in Tung of Darjeeling District, West Bengal, India. PT liquor is generally light greenish (Figure S1: C). However, it gains a light purple tint (Figure S1: D) with the addition of a few drops of lemon juice.

At first, 10g of the manufactured tea was taken, rinsed thoroughly with distilled water, air-dried, and finally suspended in 30 ml of methanol. It was then placed under a magnetic stirrer for 48 hours. The extract was then filtered using a Whatman filter paper, and the filtrate was centrifuged at 10,000 rpm for 10 minutes. The clear supernatant was again passed through Whatman filter paper, and the clear filtrate and working concentration (1mg/ml) were stored in vials at 4˚C for future use.

2.1.4. Antioxidant analysis.

2.1.4.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

Blois [8] mentioned the use of DPPH to determine the antioxidant activity of various compounds extracted using different solvents. The antioxidant compound scavenges the unpaired electron of the stable free radical of DPPH by donating a hydrogen atom. A total of five different concentrations (30µg/ml, 50 µg/ml, 70 µg/ml, 90 µg/ml, and 110 µg/ml) of PT extracts (100 µl) were mixed with 1900 µl of DPPH (dissolved in methanol). The mixture was kept in the dark and incubated for 30 minutes at room temperature. The absorbance was measured at 520 nm employing a spectrophotometer, and the radical scavenging activity of the sample was compared against the standard ascorbic acid taken in 5 different concentrations as mentioned above (30µg/ml- 110 µg/ml). The total scavenging activity was calculated using the following equation:

DPPH scavenging (%) =Acont-Asamp/Acont X 100

where Acont is the absorbance of the control (methanol and DPPH); Asamp is the absorbance of the sample mixed with DPPH. All the reactions were performed in triplicates, and the results were expressed as mean ± standard deviation (S.D.).

2.1.4.2. Ferric reducing antioxidant power assay.

Ferric reducing power assay, another determinant of antioxidant activity, was done following the protocol given by Aiyegoro [9] with some modifications. The antioxidant activity of the sample is marked by the reducing capability of the compound, which reduces the Fe3+/ferricyanide complex to the ferrous form. 250 µl of the sample of different concentrations (30 µg/ml, 50µg/ml, 70 µg/ml, 90 µg/ml, and 110 µg/ml) was first mixed with 625 µl of sodium phosphate buffer (0.2M, pH 6.6) and 625 µl of K_2Fe (CN)6 1(% w/v) and incubated at 50°C for 20 minutes. After cooling, 625 µl of TCA (10%) was pipetted into the reaction mixture and centrifuged at 3000 rpm for 10 minutes. A clear supernatant (625 µl) was diluted with an equal volume (625 µl) of distilled water. To it, 125 µl of $FeCl_3$ (0.1% w/v) was added, and the absorbance was monitored at 700nm. A higher absorbance value indicated higher reducing power. Butylated hydroxytoluene (BHT) was taken as a standard, and the ferric-reducing power was monitored in a similar concentration as of sample (30-110 µg/ml).

2.1.5. Quantitative estimation of total flavonoids.

The total flavonoids were quantified using the $AlCl_3$ method [10] with few modifications. A reaction mixture was prepared by pipetting 250µl of the sample (110 µg/ml), 750µl of deionized water, and 75µl of 5% $NaNO_2$. Following 5 minutes of incubation (RT), 150µl of $AlCl_3$ (10 %) was added and again incubated for 6 minutes (RT). 500µl of 1mM NaOH and 275µl of deionized water were added and were subsequently incubated for 30 minutes (RT). The absorbance was recorded at 510 nm using a spectrophotometer. Here, Quercetin was taken as a standard, and the total flavonoid (mg QE/g) content was calculated considering the calibration curve of Quercetin (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml) taken as a standard. The data were expressed as the mean of triplicates ± standard deviation.

2.1.6. Quantitative estimation of total phenol.

The total phenolic content of the PT sample was done following the slightly modified protocol previously given by Folin and Ciocalteu [11]. To 100 µl of the sample (110µg/ml), 400 µl of 10 % diluted Folin reagent (9:1) was added. The mixture was incubated at room temperature and in dark conditions for about 5 minutes, following which 1 ml of 5% Na_2CO_3 and again incubated for 2 hrs in dark conditions (RT). The absorbance was monitored at 730 nm employing a spectrophotometer. Here the total phenol content (mg GAE/g) was calculated considering a calibration curve of Gallic acid (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml) taken as a standard. The results were expressed as the mean of triplicates ± standard deviation.

2.1.7. Gas chromatography-mass spectrometry analysis (GC-MS).

The methanol extracts of the PT sample were further used for GC-MS analysis. The GC-MS analysis was performed in AIRF-JNU employing a GCMS-QP2010 Plus (Shimadzu Corporation, Kyoto, Japan), the details of which are provided elaborately in previous reports [12]. Here, an Rtx 5 MS capillary column was used to separate the chemicals present in the solvent extracts (Restek Company, Bellefonte, USA). The split mode was employed at a 10:1 ratio. The temperature of the injector in split injection mode was set to 250°C which was gradually ramped up to 280°C at a rate of 10°C/min after being configured to start at 100°C for 3 minutes (19 min hold). Helium was used as a carrier gas with a linear flow velocity of 40.9 cm/s. A total of 1.0 L of the solvent extract was administered, and the components present in the extract were identified by comparing their retention indices (RI) to homologous alkane series and their mass spectral fragmentation patterns to data from libraries such as NIST.LIB, NIST08.LIB, NIST08s.LIB, and WILEY8.LIB. The assumption or identification of the compounds is based on a good match between the mass spectrum and the RI.

2.2. Polypharmacological studies.

2.2.1. Target prediction of constituent phytochemicals.

Protein homology-based target prediction of the constituent phytochemicals of PT was carried out using the SwissTargetPrediction web tool (http://www.swisstargetprediction.ch/) [13] in an approach similar to our previous work [14]. The structure and SMILES formula of the compounds were extracted from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/search/search.cgi) [15]. The SMILES (Simplified

Molecular Input Line Entry System) formula is a chemical notation that allows a user to represent a chemical structure in a computer-friendly manner. These formulas of the available compounds were uploaded in SwissTargetPrediction, with "*Homo sapiens*" as our target organism. The targets that were predicted with a probability score ≥80% were considered for further analysis.

2.2.2. Drug-likeliness and Pharmacokinetic analysis.

ADME analysis of phytocompounds was conducted to identify candidates with the best chance to become effective drugs. This analysis gives an idea regarding the absorption, distribution, metabolism, and excretion of a compound in/from the human body. These properties significantly influence the performance of the compound as a drug. Hence, we submitted the SMILES formula of the concerned compounds to SwissADME, a web server that predicts the drug-like nature and ADME parameters of bioactive compounds (http://www.swissadme.ch/) [16].

2.2.3. Protein-protein interaction (PPI) and network construction.

Multiple proteins in our body interact with each other and form biological networks that regulate different metabolic processes. Plant extracts normally act by regulating several proteins involved in these biological networks. STRING Database v11.0 (https://string-db.org/) [17] helps obtain Target-Target interaction networks and their interaction with other human proteins. Thus, to understand the mechanism of action of PT, the list of probable targets obtained from SwissTargetPrediction was uploaded to the STRING Database with an interaction confidence level set to 0.9. We set the maximum number of interactions in the second shell to 50 to reduce the probability of obtaining false results (data available on request). The obtained PPI network was imported to Cytoscapev3.8.2, which helps in the visualization and analysis of biomolecular interaction networks [18]. The Network analysis includes calculating various topological features such as degree, a topological feature that shows the number of interactions a protein has with other members of the network [18]. Compounds with a higher degree are considered better targets as they can control several other proteins [19].

2.2.4. Gene ontology (GO) enrichment and Functional annotation analysis.

GO enrichment and Functional annotation analysis help to understand the biological activities of the obtained gene lists in an experiment [20]. The probable target proteins of PT were fed to DAVID Bioinformatics Resources v6.8 (https://david.ncifcrf.gov/tools.jsp) [20], which provides a functional interpretation of a large gene list. We conducted Disease annotation along with Gene Ontology (GO) enrichment analysis. GO enrichment analysis included Biological Process (BP), Cell Component (CC), and Molecular Function (MF). Pathway enrichment analysis was conducted based on KEGG Pathway. Only results with significant p-values (p-value ≤ 0.05) were considered.

3. Results and Discussion

3.1. Antioxidant activity and constituent phytocompounds.

The PT extract showed invariably higher antioxidant potential in FRAP and DPPH assay when compared against the standards BHT and Ascorbic acid (Figure 1, Table S1). The

total phenol and flavonoid in the extract were quantified as 19.36 ± 0.44 mg GAE/g and 571.801 ± 1.44 mg QE/g of extract, respectively. Previous reports of a positive correlation between the anthocyanin content and antioxidant activity of the Zijuan tea signify the direct role of anthocyanin content in the antioxidant activity of the tea [1]. This highly antioxidant nature of anthocyanin and other phytoconstituents of PT leaves may help treat chronic diseases caused by stress.

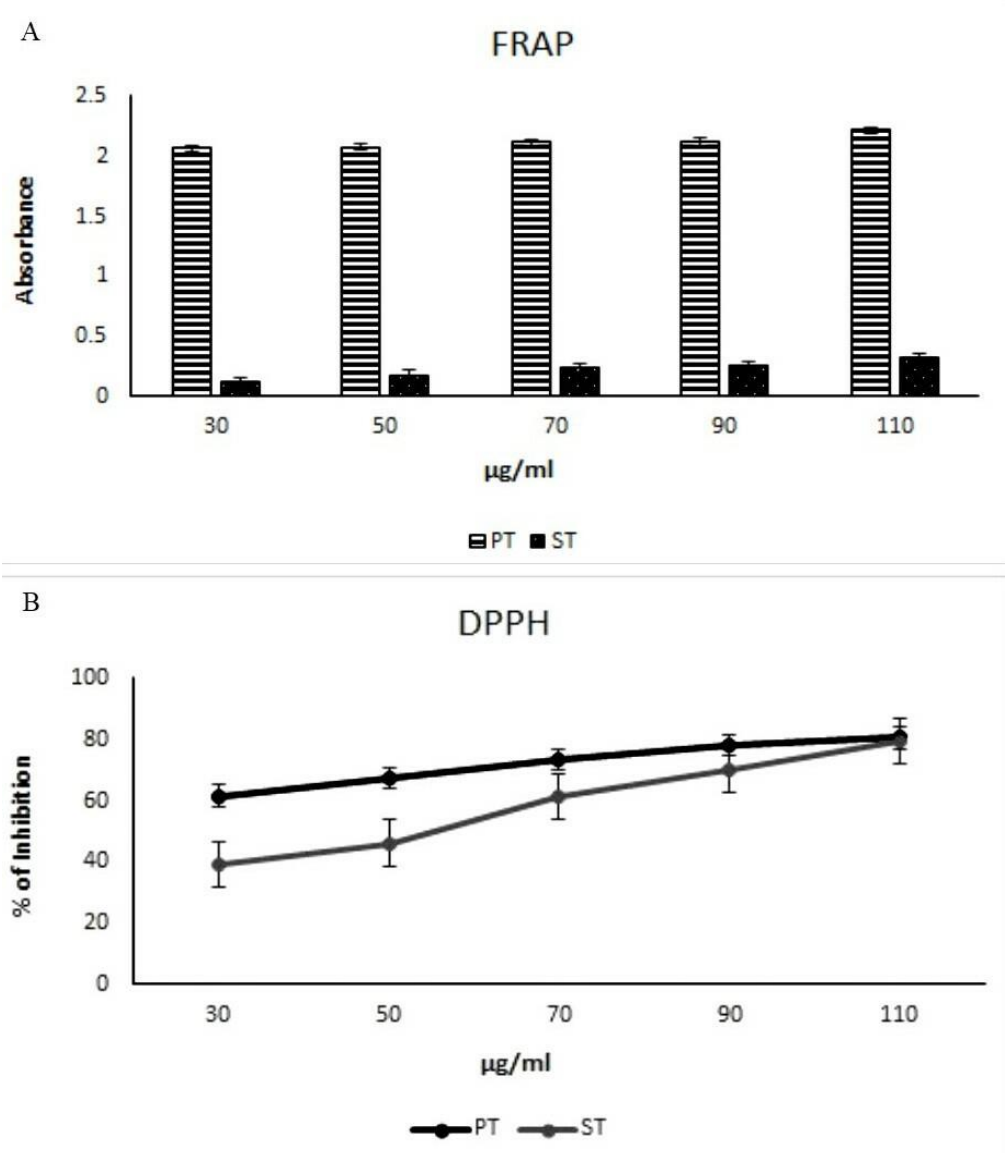

Figure 1. (A) DPPH scavenging activity of Purple tea (PT) compared against ascorbic acid used as standard (ST). **(B)** Ferric reducing antioxidant power assay of Purple tea (PT) and BHT used as standard (ST).

GC-MS identified a total of 29 constituent compounds mostly comprising hydrocarbons or their derivative, diterpene, triterpene, a vitamin derivative, long-chain fatty acids, etc. (Table S2, Figure S2). The highest peak was recorded for Coffeine (Caffeine) (60.99%), followed by 7-methoxy-2,3,6-triazaphenothiazin-1(2H)-ON (13.39%), 1,2,3 – Benzenetriol (5.01%), γ-Sitosterol (2.08%), etc.

3.2. Drug likeliness assessment.

Lipinski's rule of five, Veber's rule, etc., are the parameters implemented to assess the 'drug-likeliness' of a given compound in SwissADME [16]. The ADME properties of the

compounds are given in Table S3. The compounds hexadecanoic acid and 1,2,3-benzenetriol show blood-brain barrier (BBB) permeability, and all the compounds except γ-Sitosterol show high gastrointestinal (GI) absorption. However, none of the compounds are a substrate of permeability glycoprotein (P-gp). Another major property influencing the absorption of drugs is their solubility. In SwissADME, three topological methods are used to predict water solubility [16]. Analysis of these results shows Hexadecanoic acid to be 'moderately soluble', 1,2,3-benzenetriol and Caffeine to be 'highly soluble', and γ-Sitosterol to be 'poorly soluble'. In the case of the Bioavailability score (probability of a compound to have at least 10% oral bioavailability in rat or measurable Caco-2 permeability) of the compounds, Hexadecanoic acid shows the highest result (0.85). Thus, it can be concluded that all these compounds have the potential to be drug candidates.

3.3. Human proteins interacting with Purple tea-derived compounds.

19 human proteins were found to interact directly with the PT-derived compounds (Table 1, Figure S3). We analyzed the interaction network of all these targets and the interacting proteins obtained from STRING through Cytoscape software. The network analysis revealed EGFR (degree 35) and FYN (degree 25) have the highest degree. The other targets with a degree more than 20 were EGF (24), GRB2 (23), ERBB2 (22), and KRAS (21) (Figure 2, Table S4). This displays that the biological activity of PT may be primarily through the modulation of these targets. Epidermal growth factor (EGF)-related peptides bind with the ErbB receptors leading to the activation of intrinsic kinases [21]. These Fyn-kinases phosphorylate or dephosphorylate several target proteins and regulate the cascading mechanism of cell-cell interaction [22]. In our further analysis, FYN was found to be involved in almost all therapeutic activities of PT.

Table 1. Target proteins associated with PT and their probability of interaction.

Compound	Target	Common Name	Uniport ID	Probability
1,2,3-Benzenetriol	Tyrosine-protein kinase FYN	FYN	P06241	0.91
	Epidermal growth factor receptor erbB1	EGFR	P00533	0.91
	Carbonic anhydrase II	CA2	P00918	0.91
	Carbonic anhydrase I	CA1	P00915	0.91
	Carbonic anhydrase VI	CA6	P23280	0.91
Caffeine	Acetylcholinesterase	ACHE	P22303	0.85
	HERG	KCNH2	Q12809	0.85
	Adenosine A2a receptor	ADORA2A	P29274	0.85
γ-Sitosterol	Nuclear receptor ROR-gamma	RORC	P51449	0.92
	Niemann-Pick C1-like protein 1	NPC1L1	Q9UHC9	0.92
	LXR-alpha	NR1H3	Q13133	0.92
	Sterol regulatory element-binding protein 2	SREBF2	Q12772	0.83
	HMG-CoA reductase	HMGCR	P04035	0.83
Hexadecanoic Acid	Fatty acid-binding protein adipocyte	FABP4	P15090	0.94
	Peroxisome proliferator-activated receptor alpha	PPARA	Q07869	0.94
	Fatty acid-binding protein muscle	FABP3	P05413	0.94
	Fatty acid-binding protein epidermal	FABP5	Q01469	0.94
	Peroxisome proliferator-activated receptor delta	PPARD	Q03181	0.94
	Fatty acid-binding protein intestinal	FABP2	P12104	0.94

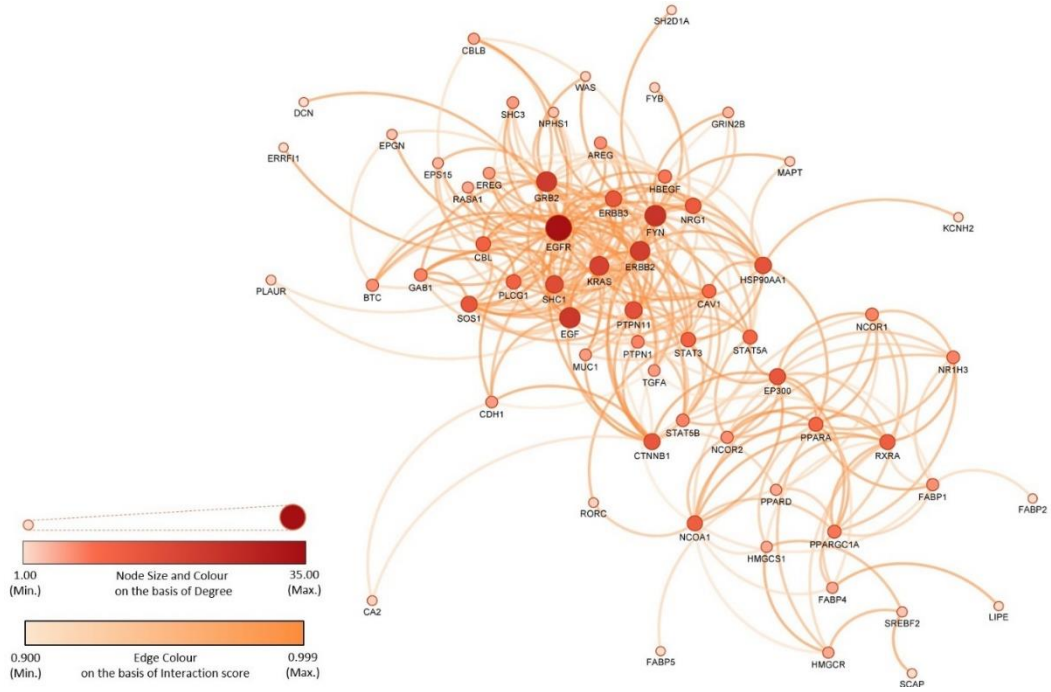

Figure 2. PPI network of PT-associated genes. The size and color of the nodes are indicative of their degree (a measure of their interaction with neighboring proteins). The edge color indicates the interaction confidence between the proteins. Darker the color higher the confidence.

GO analysis of the target genes shows them to be associated with 152BP, 62MF, and to be part of 32CC with a p-value < 0.05. The top 15 GO results obtained based on gene count are shown in Figure 3. Moreover, KEGG pathway enrichment analysis shows the association of PT targets with pathways such as the ErbB signaling pathway (21 proteins), PPAR signaling pathway (8 proteins), Insulin signaling pathway (10 proteins), Ras signaling pathway (12 proteins), Natural killer cell-mediated cytotoxicity (9 proteins), Neurotrophin signaling pathway (8 proteins), etc. These proteins are associated with different stress-induced diseases such as higher levels of plasma HDL cholesterol (Hypercholesterolemia), Type 2 diabetes, edema, coronary diseases, etc. (Figure 4).

3.4. As an immune booster and in cancer prevention.

KEGG pathway enrichment analysis of the PT targets indicated that 9 of them were associated with 'Natural killer cell-mediated cytotoxicity' in our body (Table S5). Natural killer (NK) cells, which are one of the major immune cells associated with the innate immune system, can lose their effects to kill antigens in the presence of excessive reactive oxygen species (ROS) [23]. The antioxidant nature of PT may protect the NK cells from such damage.

Moreover, antioxidant activities are always related to the induction of apoptosis and inhibition of cancer [24]. The PT interacts with the proteins associated with the 'regulation of PI-3 kinase signaling' and 'MAPK cascade' (Figure 3). These pathways have a potent role in the regulation of cell growth, cell survival, cell aging, and apoptosis [25]. FA of the targets also shows their association with cancer of Lung, Colorectal, breast, ovarian, stomach, etc. (Figure 4). Thus, PT compounds help in the overall maintenance of the normal cell cycle preventing the onset of uncontrolled cellular proliferation, i.e., cancer.

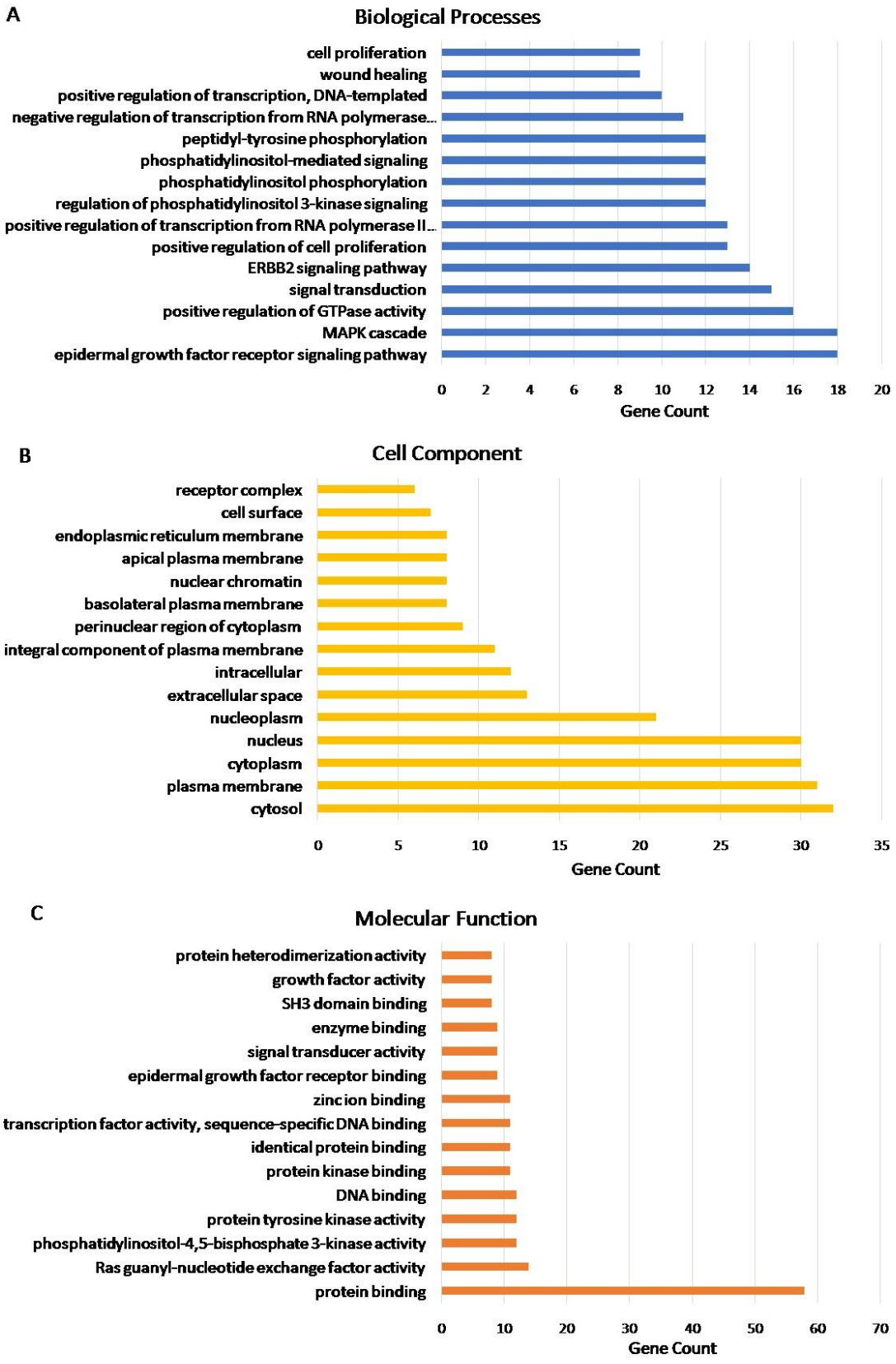

Figure 3. Gene Ontology Enrichment Analysis. The top 15 results (with p-value < 0.05) of Gene ontology enrichment analysis of the target genes show them to be associated with different (**A**) Biological Processes, (**B**) Cell Components, and (**C**) Molecular Functions.

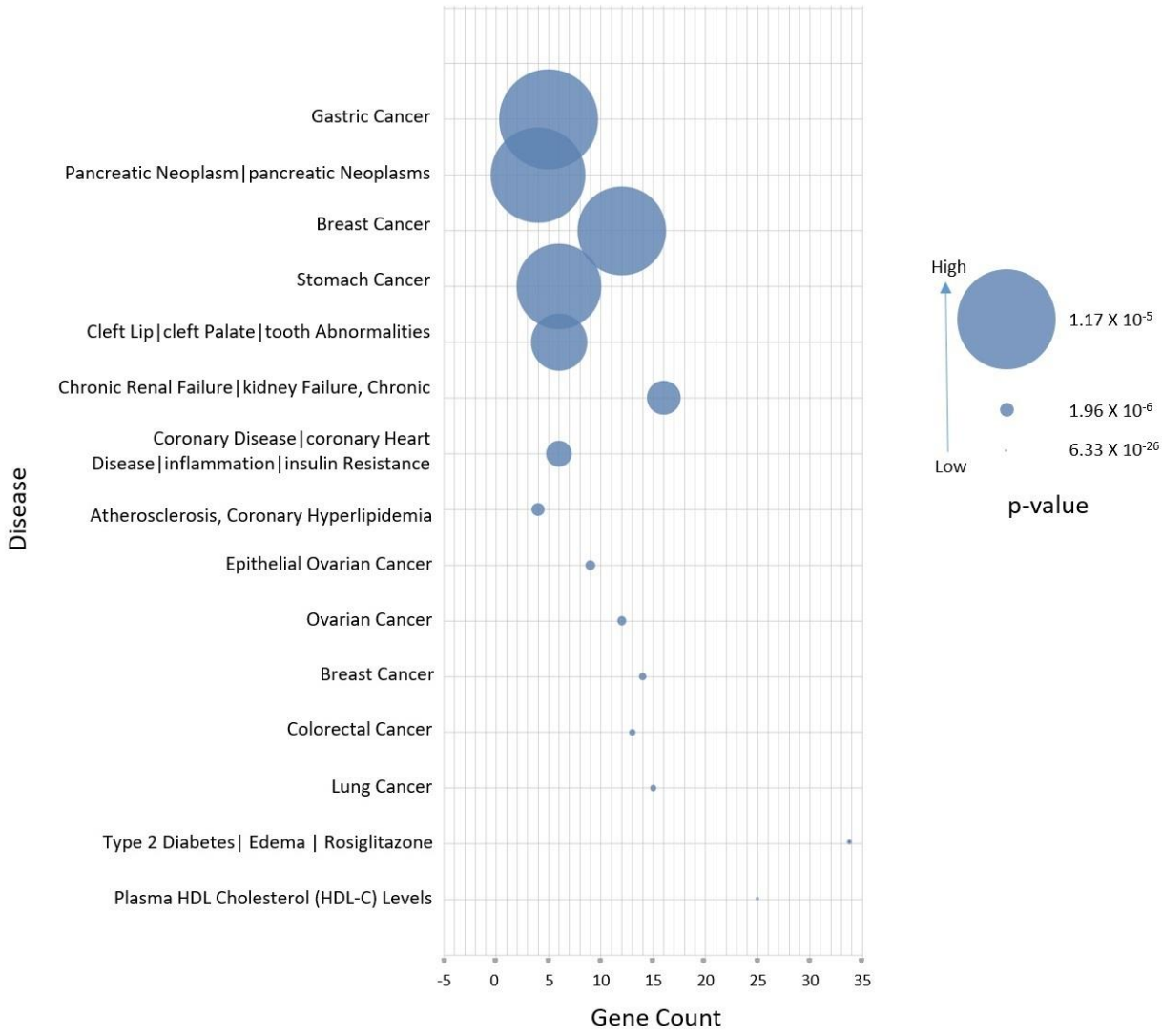

Figure 4. Functional Annotation (Disease). The top 15 results (with p-value < 0.05) of Functional annotation of PT target genes show their association with diseases such as plasma HDL cholesterol (Hypercholesterolemia), Type 2 diabetes, edema, coronary diseases, etc. The size of the bubbles indicates the p-Value

3.5. In Alzheimer's disease management.

Alzheimer's disease (AD) is a progressive neurodegenerative disease resulting in brain atrophy and the death of the brain cells affecting a person's ability to think and function independently. The phytocompounds of PT were found to be interacting with Fyn kinase via the ErbB signaling pathway. There are two major accumulations identified in AD- (a) β-amyloid (Aβ) gets accumulated as plaques, and (b) tau gets accumulated as neurofibrillary tangles (NFTs). These substantially hamper the homeostasis of the brain and neurons, leading to AD [26]. Fyn controls the functionality of the nervous system by phosphorylating different proteins associated with synaptic signal transmission. Recent studies have also revealed Fyn's importance in limiting AD occurrence by controlling tau aggregation [27].

Furthermore, FA shows five primary targets of PT compounds are associated with Alzheimer's disease (Figure 4), and they can also regulate the Neurotrophin signaling pathway (ST5). Thus, PT may maintain neural homeostasis and prevent or at least delay AD and associated neural disorders by interacting with the Fyn kinase protein.

3.6. In hyperglycemia management.

Type 2 Diabetes (T2D) is a lifelong disorder that impairs glucose metabolism and makes the patients' body insulin resistant [28]. High blood sugar (hyperglycemia) can aggravate T2D as well as be a symptom of the disease [29]. While analyzing our results, we found that 34 PT targets were associated with T2D and Edema (Figure 4). Among those 34 proteins, 11 were found to be connected with the ErbB signaling pathway, which has a role in maintaining its activity in adipocyte differentiation and insulin signaling. PT compounds may interact with FYN through the ErbB signaling pathway and prevent insulin resistance. This will further help in controlling T2D. Moreover, PT is linked with 10 targets of the Insulin signaling pathway (Table S5). Proper insulin signaling will maintain glucose uptake and utilization homeostasis, preventing the risk of insulin resistance and T2D.

PT compounds also interact with 8 proteins of PPAR signaling pathway (Table S5) which is crucial for adaptive thermogenesis. The rate of gluconeogenesis increases with stress, and this, in turn, raises the blood glucose level. Adaptive thermogenesis, in turn, maintains insulin homeostasis in the presence and absence of stress. PPAR also interacts with the RAS signaling pathway, which has a direct role in insulin signaling and glucose and lipid metabolism [30]. Thus, regular intake of PT can well-maintain the blood glucose level in the body, preventing type 2 diabetes and related insulin resistance.

3.7. In hypercholesterolemia, obesity, and coronary heart disease management.

The worldwide prevalence of hypercholesterolemia, obesity, and associated coronary heart disease is increasing rapidly. Obesity is a major lifestyle disorder that is associated with cardiovascular diseases (CVD), type 2 diabetes, hypertension, etc. [31].

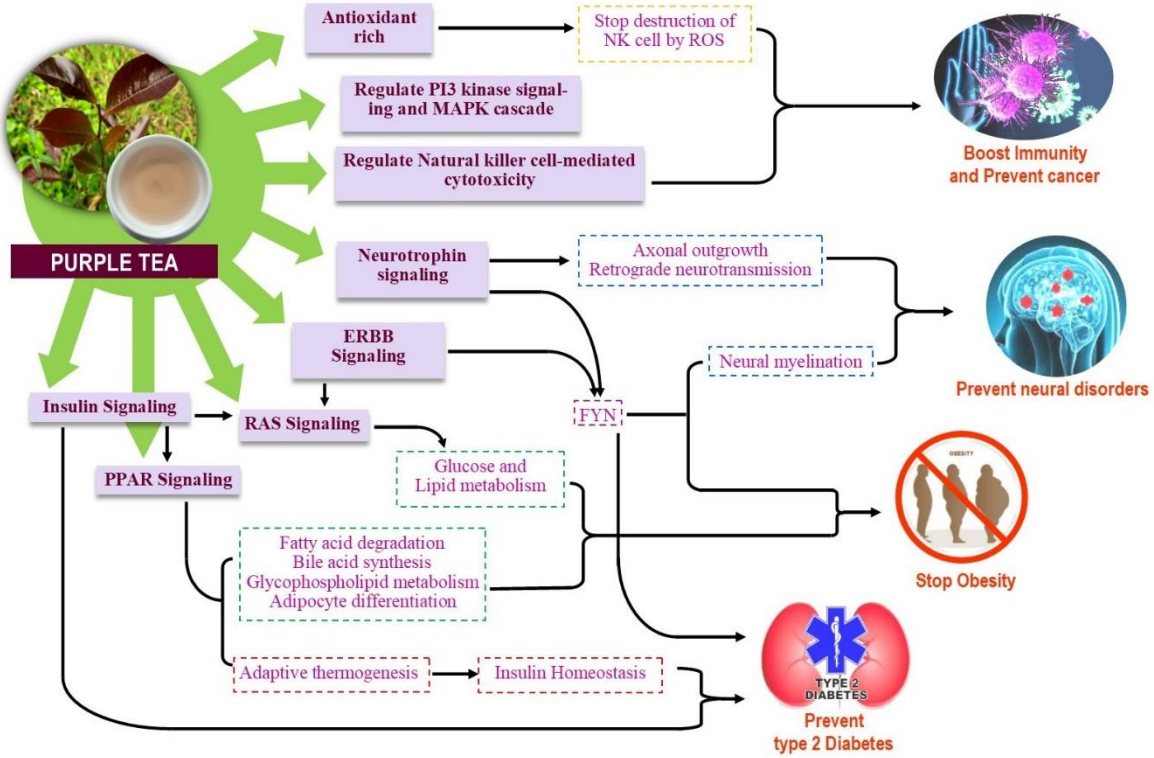

Figure 5. Schematic diagram representing the therapeutic properties of PT. PT may have potential therapeutic properties against neural disorders, hypercholesterolemia, type 2 diabetes, cancer, etc., and boost our immune system through regulating various pathways in the human body (Regarding the abbreviations**,** please refer to the main text).

Control of PT compounds over 'plasma HDL cholesterol (HDL-C) levels and 'Coronary heart disease-associated genes (25 genes and 6 genes, respectively) was evident from GO enrichment analysis and FA (Figures 3 and 4). Some studies have established the role of Fyn-kinase in obesity and related disorders [32]. The PPAR signaling pathway is also associated with lipid metabolism, adipocyte differentiation, fatty acid degradation, and bile acid synthesis [30]. Adipocyte differentiation is considered a strategy for obesity prevention and treatment [33]. Thus, we can hypothesize that the association of PT compounds with these pathways has a direct role in preventing obesity and related coronary disorders. The probable role of PT in disease management through different pathways is depicted in Figure 5.

4. Conclusions

The GC-MS analysis of PT extract provided insight into its constituent phytocompounds. From our target fishing and network pharmacology study, it was evident that human proteins that are directly interacting with the selected PT compounds are mainly associated with controlling stress-induced ailments through cell growth and survival, cell motility, immune response, axon guidance, neural transport, cholesterol metabolism, and lipid metabolism. Thus, we propose the consumption of PT that may be beneficial in promoting better health and reducing the risk of various ailments in the way of life. However, further clinical studies are needed to validate these findings.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

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Supplementary Material

Table S1. DPPH inhibition percentage of ascorbic acid (ST) and Purple tea extract (PT).

Sl. No.	Concentration (µg/ml)	PT (% of inhibition)	ST (%of inhibition)
1	30	61.60	38.96
2	50	67.59	46.22
3	70	73.34	61.27
4	90	78.14	69.83
5	110	80.52	79.20

Table S2. List of compounds characterized by GC-MS of PT.

Peak#	R.time	Area	Area%	Name
1	13.108	1707360	5.01	1,2,3-benzenetriol
2	16.968	154335	0.45	Neophytadiene
3	17.224	37841	0.11	3,7,11,15-tetramethyl-2-hexadecen-1-ol
4	17.375	20770300	60.99	Coffeine
5	17.883	681456	2	Cyclopentanetridecanoic acid, methyl est
6	19.518	42029	0.12	10,12-hexadecadien-1-ol
7	19.578	291133	0.85	9-octadecenoic acid (z)-, methyl ester
8	19.693	75125	0.22	Phytol
9	21.22	62679	0.18	Fumaric acid, 2-dimethylaminoethyl octadecyl ester
10	21.337	164596	0.48	Chloromethyl 2-chlorododecanoate
11	21.498	60047	0.18	Hexadecanoic acid
12	21.554	47262	0.14	Oxazole, 2,2'-(1,4-butanediyl)bis[4,5-dihydro-
13	22.627	29738	0.09	Petroselinic acid, tbdms derivative
14	22.677	92796	0.27	Bis(2-(dimethylamino)ethyl) ether
15	22.82	229857	0.67	Glycidyloleate
16	23.02	240975	0.71	Hexanoic acid, octadecyl ester
17	23.272	273553	0.8	Di-n-octyl phthalate
18	24.367	461379	1.35	(14z)-14-tricosenyl formate #
19	25.543	42003	0.12	Squalene
20	25.62	1627200	4.78	8-methoxy-11-methyl-11h-indolo[3,2-c]quinoline, 5-oxide
21	26.14	247977	0.73	2-(3-methoxy-5-methyl-benzylidene)-7-methy
22	27.102	75379	0.22	1,3-benzenedicarboxaldehyde, 4-hydroxy-5,6-dimethoxy-
23	27.836	178786	0.52	3-dimethylamino-6-nitro-4-phenyl-quinolin-2-ol
24	28.13	4560247	13.39	7-methoxy-2,3,6-triazaphenothiazin-1(2h)-on
25	28.497	136001	0.4	.gamma.-tocopherol
26	28.755	257605	0.76	Pyrazine, tetrakis(1-methylethyl)-
27	29.069	410371	1.2	1-(4-methoxybenzyl)isoquinoline
28	29.739	391353	1.15	Vitamin E
29	33.661	707104	2.08	gamma.-sitosterol

Table S3. ADME analysis of phytocompounds with ≥80% target prediction.

Molecule	hexadecanoic acid	1,2,3-benzenetriol	coffeine	gamma-sitosterol
Formula	C16H32O2	C6H6O3	C8H10N4O2	C29H50O2
MW	256.42	126.11	194.19	430.71
#Heavy atoms	18	9	14	31
#Aromatic heavy atoms	0	6	9	0
Fraction Csp3	0.94	0	0.38	0.93
#Rotatable bonds	14	0	0	7
#H-bond acceptors	2	3	3	2
#H-bond donors	1	3	0	2
MR	80.8	32.51	52.04	134.39
TPSA	37.3	60.69	61.82	40.46

Molecule	hexadecanoic acid	1,2,3-benzenetriol	coffeine	gamma-sitosterol
iLOGP	3.85	0.97	1.79	4.91
XLOGP3	7.17	0.52	-0.07	8.12
WLOGP	5.55	0.8	-1.03	7
MLOGP	4.19	0.18	0.22	5.8
Silicos-IT Log P	5.25	0.43	-0.5	6.44
Consensus Log P	5.2	0.58	0.08	6.45
ESOL Log S	-5.02	-1.44	-1.48	-7.16
ESOL Solubility (mg/ml)	2.43E-03	4.55E+00	6.50E+00	2.95E-05
ESOL Solubility (mol/l)	9.49E-06	3.61E-02	3.35E-02	6.86E-08
ESOL Class	Moderately soluble	Very soluble	Very soluble	Poorly soluble
Ali Log S	-7.77	-1.37	-0.78	-8.83
Ali Solubility (mg/ml)	4.31E-06	5.44E+00	3.25E+01	6.42E-07
Ali Solubility (mol/l)	1.68E-08	4.31E-02	1.67E-01	1.49E-09
Ali Class	Poorly soluble	Very soluble	Very soluble	Poorly soluble
Silicos-IT LogSw	-5.31	-0.63	-0.67	-5.62
Silicos-IT Solubility (mg/ml)	1.25E-03	2.96E+01	4.15E+01	1.05E-03
Silicos-IT Solubility (mol/l)	4.88E-06	2.34E-01	2.14E-01	2.43E-06
Silicos-IT class	Moderately soluble	Soluble	Soluble	Moderately soluble
GI absorption	High	High	High	Low
BBB permeant	Yes	Yes	No	No
Pgp substrate	No	No	No	No
CYP1A2 inhibitor	Yes	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	
CYP3A4 inhibitor	No	Yes	No	No
log Kp (cm/s)	-2.77	-6.7	-7.53	-3.16
Lipinski #violations	1	0	0	1
Ghose #violations	0	3	1	3
Veber #violations	1	0	0	0
Egan #violations	0	0	0	1
Muegge #violations	1	1	1	1
Bioavailability Score	0.85	0.55	0.55	0.55
PAINS #alerts	0	1	0	0
Brenk #alerts	0	1	0	1
Leadlikeness #violations	2	1	1	2
Synthetic Accessibility	2.31	1	2.03	6.4

Table S4. PPI network analysis of Purple tea targets (Degree ≥ average, i.e. 8.76).

SL. No.	name	BetweennessCentrality	ClosenessCentrality	ClusteringCoefficient	Degree	Eccentricity	TopologicalCoefficient
1	EGFR	0.19	0.58	0.27	35	5	0.23
2	FYN	0.13	0.53	0.30	25	5	0.24
3	EGF	0.05	0.52	0.41	24	5	0.28

SL. No.	name	BetweennessCentrality	ClosenessCentrality	ClusteringCoefficient	Degree	Eccentricity	TopologicalCoefficient
4	GRB2	0.04	0.48	0.40	23	6	0.31
5	ERBB2	0.03	0.51	0.45	22	5	0.29
6	KRAS	0.09	0.53	0.41	21	5	0.27
7	SHC1	0.01	0.46	0.58	18	6	0.37
8	PTPN11	0.02	0.49	0.56	17	5	0.35
9	HSP90AA1	0.11	0.52	0.34	16	4	0.26
10	ERBB3	0.01	0.47	0.61	15	5	0.37
11	SOS1	0.00	0.45	0.71	15	6	0.42
12	CTNNB1	0.16	0.53	0.29	15	4	0.24
13	EP300	0.15	0.50	0.33	15	4	0.22
14	NRG1	0.01	0.47	0.64	14	5	0.40
15	RXRA	0.08	0.45	0.47	13	3	0.24
16	NCOA1	0.04	0.41	0.44	13	4	0.31
17	PLCG1	0.01	0.44	0.65	12	6	0.41
18	STAT3	0.04	0.50	0.47	12	5	0.33
19	CBL	0.01	0.46	0.70	12	5	0.40
20	STAT5A	0.04	0.49	0.40	11	5	0.28
21	PPARA	0.07	0.44	0.55	11	3	0.28
22	CAV1	0.00	0.46	0.67	10	5	0.39
23	HBEGF	0.00	0.44	0.64	9	5	0.43
24	PPARGC1A	0.01	0.38	0.69	9	4	0.41

Table S5. Top 25 Pathway Enrichment analysis of Purple Tea associated genes (KEGG).

SL. No.	KEGG ID	Pathway	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment
1	hsa04012	ErbBsignaling pathway	21	33.333	3.78E-25	STAT5A, STAT5B, SHC3, SHC1, EGF, GAB1, TGFA, CBLB, NRG1, CBL, AREG, EGFR, EREG, BTC, ERBB3, ERBB2, KRAS, GRB2, PLCG1, SOS1, HBEGF	56	87	6879	29.651
2	hsa05205	Proteoglycans in cancer	17	26.984	1.57E-12	CAV1, STAT3, PLAUR, GAB1, CBLB, PTPN11, CBL, DCN, EGFR, ERBB3, ERBB2, CTNNB1, KRAS, GRB2, PLCG1, SOS1, HBEGF	56	200	6879	10.441
3	hsa05200	Pathways in cancer	19	30.159	5.84E-10	STAT5A, STAT5B, HSP90AA1, EGF, STAT3, TGFA, CBLB, CBL, EGFR, RXRA, CDH1, ERBB2, EP300, CTNNB1, KRAS, GRB2, PLCG1, SOS1, PPARD	56	393	6879	5.939
4	hsa05220	Chronic myeloid leukemia	10	15.873	3.91E-09	STAT5A, STAT5B, SHC3, SHC1, CBLB, KRAS, PTPN11, GRB2, CBL, SOS1	56	72	6879	17.061

SL. No.	KEGG ID	Pathway	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment
5	hsa05223	Non-small cell lung cancer	9	14.286	1.04E-08	RXRA, EGF, ERBB2, TGFA, KRAS, GRB2, PLCG1, SOS1, EGFR	56	56	6879	19.742
6	hsa05215	Prostate cancer	10	15.873	2.38E-08	HSP90AA1, EGF, ERBB2, EP300, CTNNB1, TGFA, KRAS, GRB2, SOS1, EGFR	56	88	6879	13.959
7	hsa05214	Glioma	9	14.286	3.50E-08	SHC3, SHC1, EGF, TGFA, KRAS, GRB2, PLCG1, SOS1, EGFR	56	65	6879	17.009
8	hsa05213	Endometrial cancer	8	12.698	1.43E-07	CDH1, EGF, ERBB2, CTNNB1, KRAS, GRB2, SOS1, EGFR	56	52	6879	18.898
9	hsa05100	Bacterial invasion of epithelial cells	9	14.286	1.50E-07	SHC3, SHC1, CDH1, CAV1, GAB1, WAS, CTNNB1, CBLB, CBL	56	78	6879	14.174
10	hsa03320	PPAR signaling pathway	8	12.698	8.47E-07	FABP1, FABP2, RXRA, FABP4, FABP5, NR1H3, PPARA, PPARD	56	67	6879	14.667
11	hsa04910	Insulin signaling pathway	10	15.873	1.18E-06	LIPE, PTPN1, SHC3, SHC1, CBLB, KRAS, GRB2, CBL, SOS1, PPARGC1A	56	138	6879	8.901
12	hsa04014	Ras signaling pathway	12	19.048	1.26E-06	SHC3, SHC1, EGF, RASA1, GAB1, KRAS, PTPN11, GRB2, PLCG1, SOS1, GRIN2B, EGFR	56	226	6879	6.522
13	hsa04520	Adherens junction	8	12.698	1.26E-06	PTPN1, CDH1, ERBB2, WAS, EP300, CTNNB1, FYN, EGFR	56	71	6879	13.841
14	hsa04917	Prolactin signaling pathway	8	12.698	1.26E-06	STAT5A, STAT5B, SHC3, SHC1, STAT3, KRAS, GRB2, SOS1	56	71	6879	13.841
15	hsa04650	Natural killer cell mediated cytotoxicity	9	14.286	4.71E-06	SHC3, SHC1, SH2D1A, KRAS, PTPN11, GRB2, FYN, PLCG1, SOS1	56	122	6879	9.062

SL. No.	KEGG ID	Pathway	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment
16	hsa05221	Acute myeloid leukemia	7	11.11111	4.72E-06	STAT5A, STAT5B, STAT3, KRAS, GRB2, SOS1, PPARD	56	56	6879	15.35491
17	hsa05160	Hepatitis C	9	14.28571	8.96E-06	RXRA, EGF, STAT3, NR1H3, KRAS, GRB2, PPARA, SOS1, EGFR	56	133	6879	8.312433
18	hsa04915	Estrogen signaling pathway	8	12.69841	1.19E-05	HSP90AA1, SHC3, SHC1, KRAS, GRB2, SOS1, EGFR, HBEGF	56	99	6879	9.926407
19	hsa05211	Renal cell carcinoma	7	11.11111	1.24E-05	GAB1, EP300, TGFA, KRAS, PTPN11, GRB2, SOS1	56	66	6879	13.02841
20	hsa05219	Bladder cancer	6	9.52381	1.63E-05	CDH1, EGF, ERBB2, KRAS, EGFR, HBEGF	56	41	6879	17.97648
21	hsa04510	Focal adhesion	10	15.87302	3.13E-05	SHC3, SHC1, EGF, CAV1, ERBB2, CTNNB1, GRB2, FYN, SOS1, EGFR	56	206	6879	5.963072
22	hsa04722	Neurotrophin signaling pathway	8	12.69841	4.18E-05	SHC3, SHC1, GAB1, KRAS, PTPN11, GRB2, PLCG1, SOS1	56	120	6879	8.189286
23	hsa05231	Choline metabolism in cancer	7	11.11111	1.40E-04	EGF, WAS, KRAS, GRB2, PLCG1, SOS1, EGFR	56	101	6879	8.513614
24	hsa05212	Pancreatic cancer	6	9.52381	1.56E-04	EGF, ERBB2, STAT3, TGFA, KRAS, EGFR	56	65	6879	11.33901
25	hsa04919	Thyroid hormone signaling pathway	7	11.11111	2.85E-04	NCOA1, RXRA, NCOR1, EP300, CTNNB1, KRAS, PLCG1	56	115	6879	7.477174

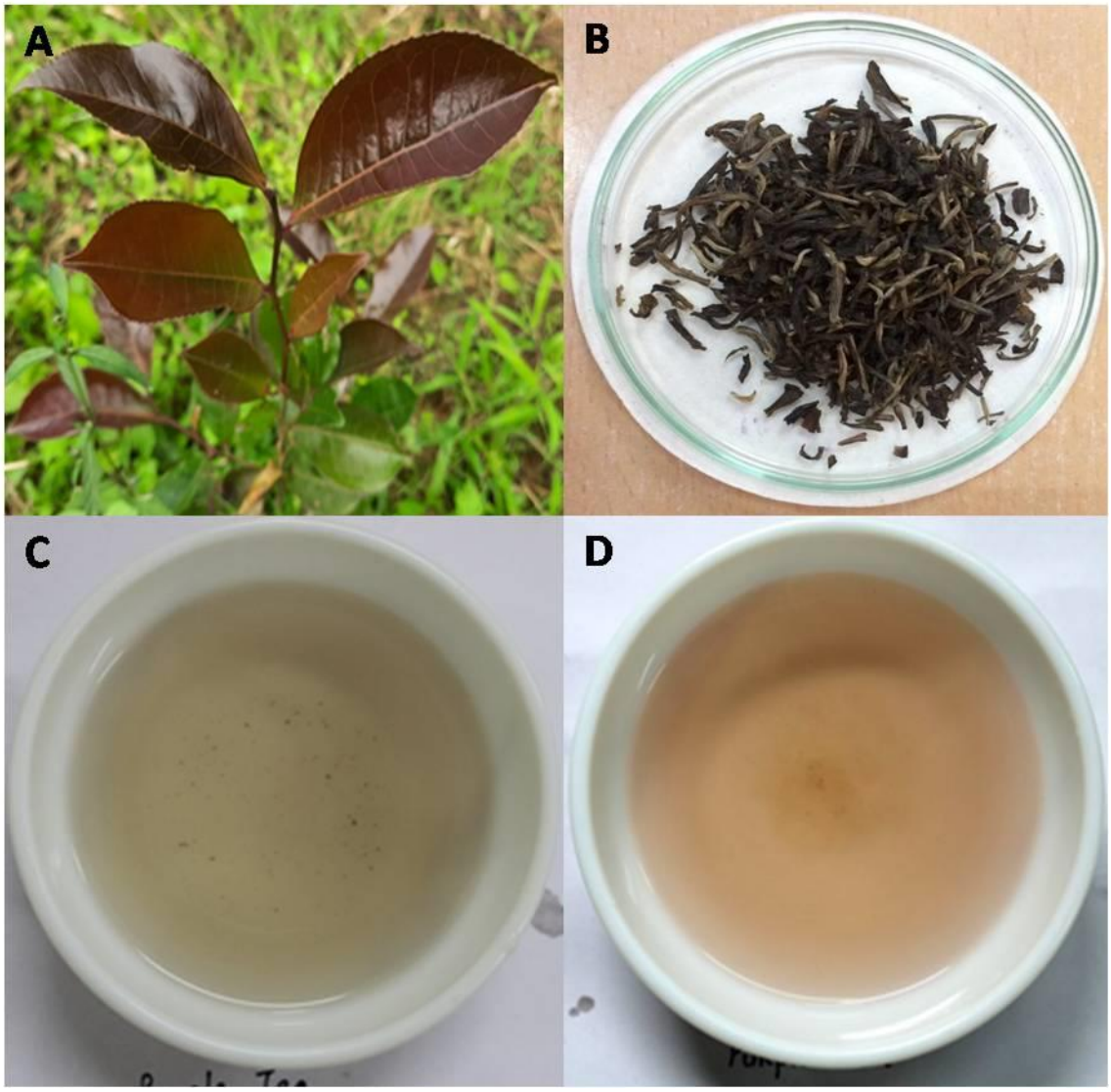

Figure S1. (A) PT plant. (B) Processed PT (C) PT extract without lemon. (D) PT extract with few drops of lemon (adding lemon gives the tea a purple tint).

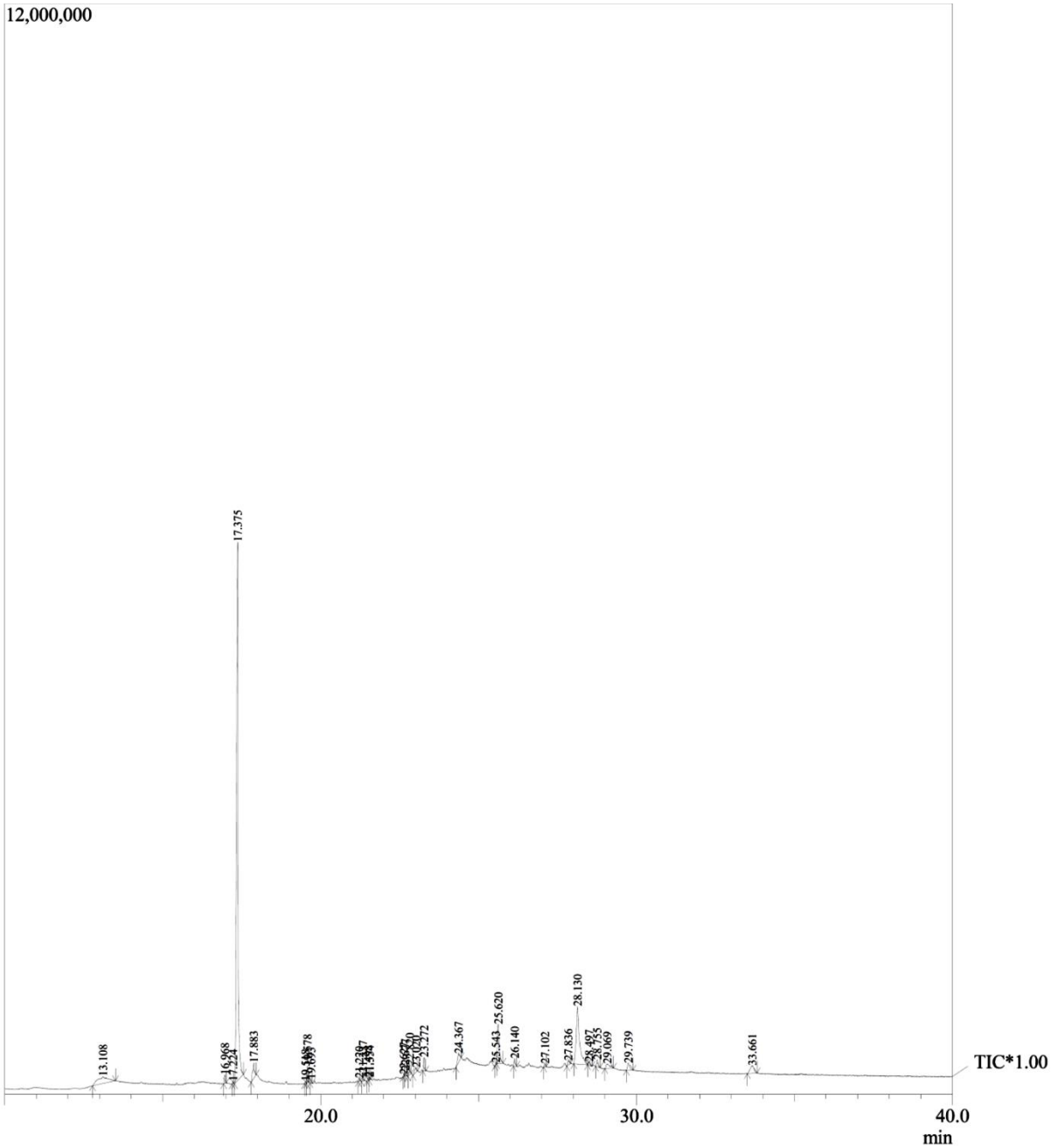

Figure S2. GC-MS Chromatogram of PT showing the peaks for constituent phytomolecules and their retention time.

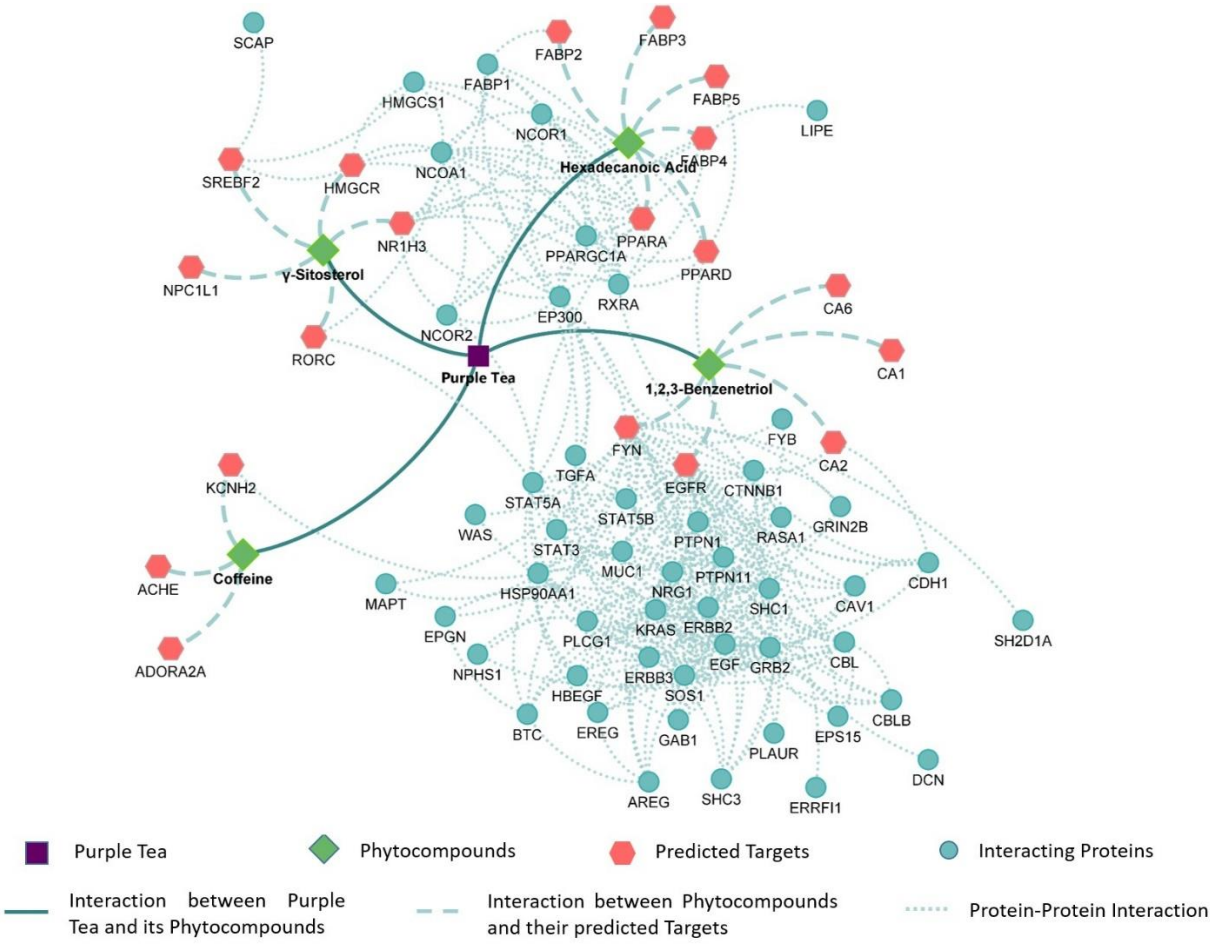

Figure S3. Phytocompound-target interaction and PPI network of PT-associated proteins. The four significant phytocompounds found in purple tea are shown along with their target proteins. The network between these target proteins and other human proteins is also depicted.