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BLACK TEA THEAFLAVINS: A REVIEW OF THE EVIDENCE

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ABSTRACT: Tea brewed from the plant *Camellia sinensis* is the second most popular beverage consumed worldwide after water. The first two leaves and the leaf bud are mainly used for commercial tea production, and depending on the variation of processing techniques, it is primarily categorized into -white tea, green tea, oolong tea, and black tea. Different varieties of tea are known for their contribution in little to a large amount of bioactivity or sensory attributes due to variation in the chemical constituents, thus providing health benefits. Throughout the years green tea has been extensively investigated by researchers for its health benefits; however, black tea is now emerging as more beneficial to health and catching serious attention by researchers. Apart from the methylxanthines, amino acids, phenolics, and catechins, the two major components, *i.e.*, the TFs and TRs are solely responsible for black tea's characteristic color and taste and are proven to have health benefits. This review aims to provide available information on Theaflavins (TFs), their bioactivity, and, most importantly, the various methods for the extraction of TFs of black tea.

INTRODUCTION: Tea is one of the most consumed aromatic beverages in the world and is produced on a large scale in more than 30 countries, primarily in China, Japan, India and Sri Lanka [1]. The first two leaves and the leaf buds of the tea plant *Camellia sinensis*, belonging to the genus *Camellia* under the family Theaceae of the flowering plants, are mainly used for the production of commercial tea. There are mainly three varieties of tea species found in India - China variety *i.e., Camellia sinensis ssp. sinensis*, Assam variety *i.e. C. assamica* ssp. *Assamica* and Cambod variety *i.e. C. assamica ssp. lasiocalyx*. There are various flushes of tea depending on the plucking season of the leaves *i.e.*, the first flush usually occurring between March-April, the second flush usually harvested between late May-June, and the third flush harvested between October-November.

The first flush is said to be the best among three in quality, appearance, and taste as the tea plants go through a dormant phase after the last harvest, *i.e.*, the third flush; thus in the fresh flush of next season there exist higher amount of aromatic compounds making a huge difference in the chemical compositions compared to the other two flushes. The variations in the processing technique for commercial tea production involve a different degree of oxidation, traditionally known as "fermentation" of the leaves, stopping oxidation, forming the tea, and drying it. Depending on the degree and period of oxidation and the

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characteristic quality of tea, it is categorized into four main types - the non-oxidized white tea from the leaf buds, the non-oxidized green tea, the partially oxidized oolong tea, and the fully oxidized black tea [2]. Among all the four types of tea, black tea occupies 80% of the world's tea production while the rest is contributed by the others [3]. The oxidation process of the tea leaves is traditionally known as the fermentation process, which is very much different from the anaerobic breakdown of energy-rich compounds such as carbohydrates to alcohol or organic acids using microorganism but is mainly the oxidative polymerization and condensation of the Flavan-3-ols catalyzed by the endogenous polyphenol oxidases [2].

The manufacturing process of black tea in its orthodox form goes through four different stages *i.e.*, withering, rolling, "fermenting," and firing, as a result of which only 15% of the catechins from green tea remains unchanged, whereas the rest of the catechins (Flavan-3-ols) are polymerized into Theaflavins (TFs) and Thearubigins (TRs). The parental composition of black tea strongly resembles the other teas but differs by having the two major polyphenols, *i.e.*, the TFs and TRs, which are solely responsible for the color and the taste of it. Depending on the presence of the oxidation products like TFs and TRs, the characteristic qualities, including aroma, taste, color, and appearance of different tea types, are determined.

The TFs are dimeric polyphenols possessing a benzotropolone skeleton with dihydroxy or trihydroxy aromatic moieties as substituents and a characteristic yellow-orange color comprising 3-6% of the dry weight of black tea [4]. Epidemiological investigations on the consumption of black tea have proven to have numerous health-beneficial attributes, including anti-microbial, antiviral, anti-diabetic, anti-cancerous activities as well as conferring neuroprotective effects [5]. The TFs individually have been proven to have acetylcholine esterase inhibition properties in mice models [6]. Due to the presence of some different major bioactive components in black tea and their beneficiary effects on health, it is currently a new hot topic, and the extraction of the various tea components has become a common interest among researchers. Various extraction methods are performed for the extraction of tea biomolecules which involves. 1 Conventional solvent extraction method for the catechins and caffeine where different solvents are used for the extraction of these bioactive components using the classical techniques like Soxhlet extraction, maceration, and Hydrodistillation [7]. 2 Ultrasound-assisted extraction method [UAE] for tea catechins and proteins where the mechanism involves ultrasound intensification of mass transfer and accelerated access of solvent to cell materials of plant parts [8]. 3 Microwave-assisted extraction [MAE] method for the extraction of polyphenols, polysaccharides, and cut in where extraction is done by using a wide range of microwave energy occurring in three sequential steps- separation of solutes from active sites of sample matrix using high temperature and pressure; diffusion of solvent across sample matrix; release of solutes from sample matrix to solvent [9].

4 High-pressure processing [HPP] for extraction of different tea components where an adiabatic heating process is applied by exerting high pressure which facilitates the extraction process by increasing the permeability and solubility of the plant extracts [10]. 5 Supercritical fluid extraction [SFE], particularly for the extraction of caffeine for decaffeination and catechins, where the components are extracted from a packed bed adsorption column with the help of fluidized CO_2 by high pressure. In the case of SFE, the fluid that is in use is converted into a supercritical fluid by application of high temperature and pressure beyond its critical point thus; the supercritical fluid possesses lower viscosity and higher diffusivity than any other liquid [11].

In the case of isolation and extraction of the TFs and TRs, different chromatographic techniques (TLC, CC, HPLC, GC-MS & HSCCC) and non-chromatographic parameters are applied. To better understand the bioactivity and pharmacological properties of a particular component of any plant, it is essential to isolate that particular component from the plant extract so that its potentiality to become a drug can be scrutinized. In this present review, the main focus has been shifted towards the different biochemical constituents of black tea, especially emphasizing TFs, their bioactivity, and the various chromatographic and non-

chromatographic methods applied for the isolation of TFs.

Bioactive Components of Tea: The fresh tea leaves of different flushes contain on average 36% polyphenolic compounds (simple and complex polyphenols), 25% carbohydrates (pectins, glucose, fructose, cellulose), 15% proteins, 6.5% lignin, 5% minerals, and trace elements ((magnesium, chromium, iron, copper, zinc, sodium, cobalt, potassium, *etc*.), 4% amino acids (such as theanine [5-N-ethyl-glutamine], glutamic acid, tryptophan, aspartic acid), 2% lipids, 1.5% organic acids, 0.5% chlorophyll as well as carotenoids and ethereal substances below 0.1% and vitamins (B, C, E) [4] whereas a typical tea beverage containing 2.5 g of processed tea leaves brewed for 3 min in 250 ml hot water, usually contains 620-880 mg of different water-extractable solids [12].

The major constituents of tea biomolecules belong to the polyphenol group, which mainly include six groups of compounds, such as phenolic acids (PA), flavones, flavonols, anthocyanins, flavanols and hydroxyl-4 flavanols among which the most important tea polyphenols are the flavanols of which the flavan-3-ols commonly known as catechins are the predominant one [13.] The major catechins that are found both in green tea (GT) and black tea Black Tea (BT) are - (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (+)-catechin (C) and (+)-gallocatechin (GC) [14, 15]. These major catechins are mainly colorless water-soluble components contributing to the bitterness, astringency, and sweet aftertaste of tea [16].

Apart from these six major catechins the gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate, epigallocatechin gallate, 3-O-methyl EC, 3-O-methyl EGC, and some other minor catechins are present in smaller fractions [12]. The amount of catechins varies considerably in different tea varieties though the most abundant catechin found in all the varieties is EGCG, where the green tea and oolong tea have been shown to contain 30-130 mg of it per cup (237 ml) and black tea may have up to 70 mg of EGCG per cup. Depending on the oxidation process, the amount of total catechins varies, where black has shown to contain fewer amounts of catechins than green tea.

The major three flavonol aglycones reported in tea involve Quercetin, kaempferol, myricetin, occurring both as free flavonols and as flavonol glycosides making up to 0.5%- 2.5% wt/wt extract in tea infusions. The major simple polyphenols found in tea are gallic acid and its quinic acid ester, theogallin. In black tea, the amount of gallic acid is slightly higher than the other tea varieties whereas the amount of theogallin decreases due to the formation of theagallinin, which is a theaflavin type compound.

TABLE 1: VALUES ARE EXPRESSED IN MG/G OF DRY MATTER

	C	EGC	EC	EGCG	ECG	Total catechins
Black tea (Darjeeling)	0.7 ± 0.01	3.0 ± 0.05	2.3 ± 0.01	24.9 ± 0.20	5.9 ± 0.01	36.9 ± 1.8
Green tea (Indian)	1.3 ± 0.02	19.0 ± 0.09	4.4 ± 0.03	23.4 ± 0.05	5.6 ± 0.03	53.5 ± 1.5

It is a reference for the table, it is not included in the text The oxidation (fermentation) process allows the tea leaves to undergo enzymatic oxidation where the internal polyphenol oxidase causes the polymerization of the flavan-3-ols to catechin oligomers resulting in the formation of bisflavanols and the major two pigments of black tea, *i.e*., theaflavins (TFs) & thearubigins (TRs).

As a result, the percentage of catechins decreases in black tea, and the rest are transformed into TFs & TRs, contributing to the sweet aroma of malt sugar and the dark brown hue of black tea [4]. Theaflavins and thearubigins account for 3-6% and 12-18% of the dry weight of black tea whereas the methylxanthines account for 8-11% including the major one *i.e.* caffeine and small amounts of theophylline and theobromine. Tea contains many amino acids but the most abundant and major one which is specific to the tea plant is “Theanine” (5-N-ethyl-glutamine) accounting for almost 50% of its total amino acid content giving it the brothy taste and the degradation of it is the main reason behind the biogenesis of tea aroma [17].

Theaflavins: One of the most important catechin oxidation products giving rise to the major pigment of Bt is Theaflavin.

The group of TFs is mainly comprised of four major TFs and some minor TFs with their related compounds. The term theaflavins were set up by Roberts (1958) for orange-reddish pigments in tea. The structural elucidation conducted by collier *et al.,* 1973 led to TFs having a benzotropolone moiety.

The benzotropolone moiety is achieved *via* co-oxidation of selected pairs of catechins, one having a *vic-trihydroxyphenyl* moiety whereas the other possessing *ortho-* dihydroxy phenyl moiety. These two catechins combine at the B ring by an oxidative coupling *via* o-quinones [12]. There are four major TFs found in Black Tea (BT), are- Theaflavin (TF),

Theaflavin 3-monogallate (TF3G), Theaflavin 3'-monogallate (TF3'G) and Theaflavin 3, 3'-digallate (TF3,3'DG). Besides these four major TFs, stereoisomers and theaflavin derivatives including isotheaflavin, neotheaflavin, theaflavic acids and theaflavates have also been reported from black tea [19]. Reference for the table, not included in the text.

TABLE 2: EXPECTED COMPOSITION OF BLACK TEA IN SOLID EXTRACTS

Components	Total content (%)
Catechins	10-12
Flavonols	6-8
Theaflavins	3-6
Thearubigins	12-18
Phenolic acids	10-12
Amino acids	13-15
Methylxanthines	8-11
Carbohydrates	15
Proteins	1
Minerals	10

TABLE 3: PRECURSORS OF MAJOR THEAFLAVINS AND ITS DERIVATIVES

Precursor A	Precursor B	Product
EC	EGC	TF
EC	EGCG	TF3MG
ECG	EGC	TF3'MG
ECG	EGCG	TF3,3'DG
EC	GC	Isotheaflavin
C	EGC	Neotheaflavin
EC	Gallic acid	Epitheaflavic acid
C	Gallic acid	Theaflavic acid
EGC	Gallic acid	Epitheaflagalline
GC	Gallic acid	Theaflagalline
EGCG	Gallic acid	Epitheaflagalline-3-gallate

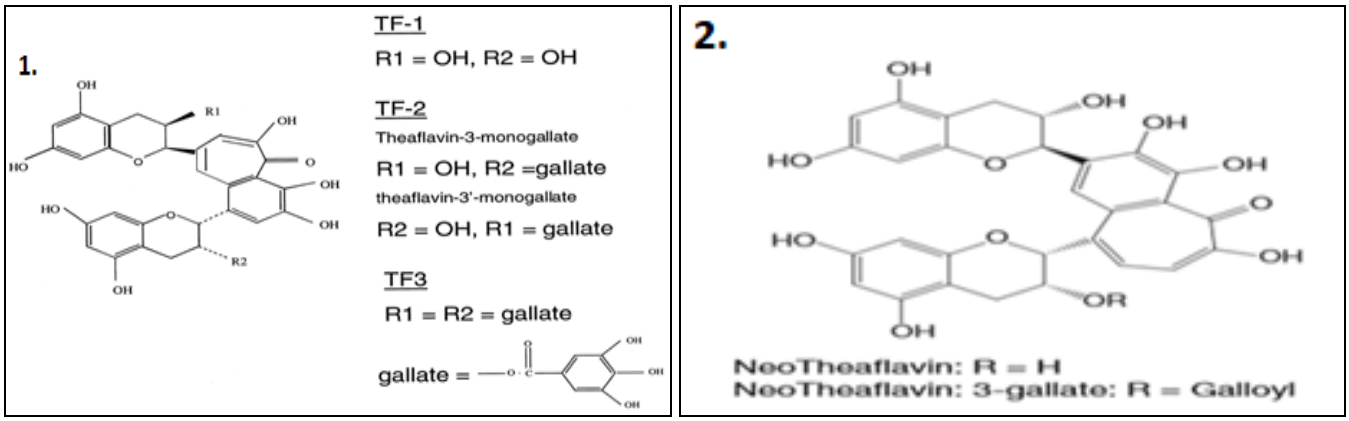


FIG 1 & 2: STRUCTURE OF MAJOR& MINOR TFS PRESENT IN BLACK TEA

Bioactivity of Theaflavin: Throughout the years the beneficial health attributes of green tea particularly the role of flavonoids and catechins of it has been studied extensively by the researchers mainly focusing on the antioxidative properties, including inhibition of generation of ROS (reactive oxygen species) as well as chelation of transition metal ions (Fe & Cu) catalyzing those reactions [20, 21]. Reactive oxygen species (ROS) are produced as a natural by-product of cellular metabolism of the living system though excessive production of ROS due to various natural stresses can cause progressive oxidative damage to the cell, which can be to DNA, Protein, and other biological macromolecules, thereby causing pathological changes to the cell eventually leading to various chronic diseases [22]. To combat those oxidative damage, antioxidants play a pivotal role in preventing or slowing down the progression of ROS formation. Though Black Tea (BT) contains a much lower concentration of monomeric catechins (C, EC, ECG, EGCG, etc.), representing only 15% of the unoxidized catechins of GT still it is a major source of dimeric and oligomeric catechins belonging to Oxytheotannins represented by TFs & TRs which has proven to contribute greatly to the

antioxidant activity of Black Tea (BT). Different antioxidative assays mainly include Ferric Ion Reducing Antioxidant Power - FRAP, Oxygen Radical Absorbance Capacity - ORAC and Trolox Equivalent Antioxidant Capacity-TEAC catechins of tea in its monomeric or epimerized form results in a transient increase in total antioxidant capacity of plasma [23, 24]. However, many epidemiological studies reported the immense health benefits of black tea and particularly theaflavin and its galloyl esters had been proven to possess several health benefits including antioxidant, anti-obesity, anticancer, anti-atherosclerotic, anti-inflammatory, antiviral, antibacterial, anti-osteoporotic, and anti-dental caries properties as well as significant effect on cognitive impairment involving inhibition of acetylcholine esterase activity.

Antioxidant Activity: An antioxidant is a compound that prevents oxidative damage by preventing the formation of ROS as well as scavenging free radicals like superoxide, H_2O_2, singlet oxygen, nitric oxide, *etc.* Studies have found that the scavenging properties of tea polyphenols mainly the catechins and their polymers on free radicals are due to the presence of an ortho-dihydroxyl group in the B ring and a galloyl moiety at the 3 position is important to maintain the effectiveness of the radical scavenging activity [25, 26]. TFs are one of Bt's most important catechin oxidation products, and it contains more hydroxyl groups than catechins because of the condensation between a catechol type B-ring of EC and a pyrogallol type B ring of EGC giving it the characteristic benzotropolone moiety [27].

The resonance form of benzotropolone moiety in TFs plays a pivotal role in scavenging free radicals *via* donation of an electron to them [28]. An *in-vitro* experiment on peroxidation of rat liver homogenate induced by tert-butyl-hydroxy peroxide (BHP) showed that the antioxidant activity of TFs of Bt is much more effective compared to glutathione (GSH), L(+)-ascorbic acid (AsA), dIN-tocopherol, butylated hydroxyl toluene (BHT) and butyl hydroxyanisole (BHA) [29]. A comparative study between the antioxidative activity of the monomeric catechins of Gt and dimeric catechins (TFs) of BT showed that TF33'DG has higher antioxidative capacity than EGCG, reacting over 10 times faster with superoxide radical than EGCG also all the catechins and TFs inhibited Cu2+ mediated LDL oxidation in the order: TF33'DG > ECG > EGCG ≥ TF3'MG ≥ TF3MG > TF≥ EC > EGC [30, 31, 32]. Apart from their free radical scavenging activity, the antioxidant activity of TFs is attributed to their ability to inhibit pro-oxidant enzymes, as a result of which they form stable complexes with metal ions like iron and copper *via* chelation thus preventing lipid peroxidation effectively by cutting off the chain reaction occurring during the oxidation of lipid [33].

The TFs suppress oxidative stress by inhibiting cytochrome P4501A1 and other oxidant enzymes under in-vitro conditions, thus preventing DNA damage [34]. To exploit the effect of TFs on the responses of human subjects to acute anaerobic interval training, a randomized, double-blind, crossover study was conducted by researchers where the results showed that consumption of Black Tea (BT) extract enriched with TF led to a reduction in oxidative stress as well as improvement in recovery and delayed the onset of muscle soreness as a response of acute anaerobic intervals [35]. TFs have also been reported to reduce oxidative stress induced by fluoride via targeting miRNA-128-3p as well as activation of the Nrf2 pathway [36].

Anti-inflammatory Activity: The different anti-inflammatory activities of TFs of black tea on animal models were evidenced by researchers using cell lines for inflammatory responses. The TFs and its galloyl esters along with EGCG have been proven to selectively affect the production of pro-inflammatory cytokines like IL-1β, TNF-α, IL-6, IL-8 where TFs have been shown to exert its anti-inflammatory response via decreasing the level of expression of cytokine IL-6 during viral infection [37]. The TF2 *i.e.* TF3MG exerted its anti-inflammatory effect by suppressing the expression of the COX-2 gene induced by 12-Otetradecanoylphorbol-13-acetate in the cell model and it also showed downregulation of TNF-α, inducible nitric oxide synthase (iNOS), ICAM-1, and nuclear factor κB (NF-κB) [38]. It has been reported that TF3'MG showed preventive effects on tumor necrosis factor superfamily 14 (TNFSF 14)-mediated IL-6 production in human gingival fibroblasts [39] as well as investigation on the effects of TF3'MG on CXC chemokine ligand 10

production from human gingival fibroblasts showed that TF3'MG prevents OSM-mediated CXCL10 production in a dose-dependent manner [40]. In case of cerebral injury, TFs conferred protection against neurons by limiting the infiltration of leukocytes as well as expression of ICAM-1 and suppressing the up-regulation of pro-oxidative enzymes like iNOS and COX-2 in the ischemic brain via reduction of phosphorylation of STAT-1 [41]. Some epidemiological investigations demonstrated the effect of TFs on the prevention of cognitive impairment precisely on Parkinson's disease where attenuation of neuronal inflammation has been reported indicating its potentiality to become a drug in the future for treating different neurodegenerative diseases [42]. In another report, TFs and TRs have been proven to inhibit acetylcholine esterase in mice models, the main enzyme working behind the progression of Alzheimer's disease [6].

Anti-periodontitis Activity: Consumption of black tea has been proven beneficial in caries development and progression. The Bt brew enriched in TFs appears to protect the supporting structures of teeth by suppressing salivary amylase activity, decreasing tooth surface pH, therefore, reducing the growth and pathogenicity of different periodontal microorganism particularly *Porphyromonasgingivalis* an anaerobic, gram-negative bacillus involved in the progression of periodontitis via destruction of the periodontal connective tissues and bone [43].

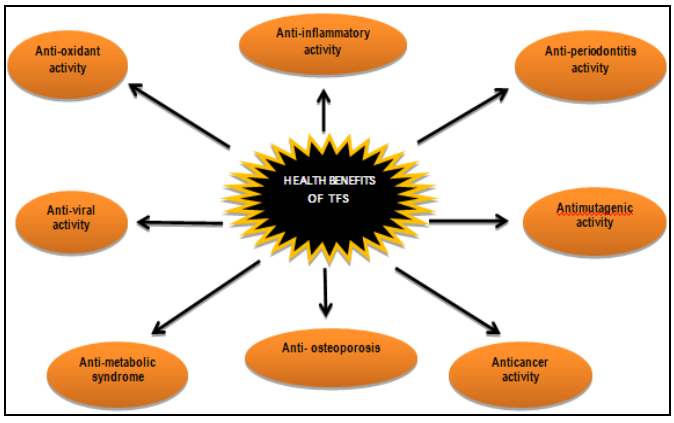
P. gingivalis has been reported to exert its pathogenic effect in the activation of matrix metalloprotease (MMP) (a proteolytic enzyme that degrades components of extracellular matrix including collagen), particularly MMP-1 (collagenase-1) and MMP-2 (gelatinase-A), as well as affecting collagen synthesis resulting in irreversible tissue destruction and progression of periodontitis [44, 45]. In an investigation, Kong and his co workers demonstrated the effects of TFs on pathogenicity of *P. gingivalis* and progression of periodontitis by inhibition of production of MMPs induced by the pathogen [46]. The Tfs exerted its antimicrobial activity against the planktonic culture and biofilm of *P. gingivalis* as well as showed inhibitory effects on the proteinase activities of *P. gingivalis* collagenase and gingipains in a dose-dependent manner. The TFs also appear to inhibit the secretion and expression of mRNA MMP-1 & MMP-2 by human gingival fibroblasts induced by *P. gingivalis* thus attenuating MMP mediated inflammatory response induced by this pathogen [46]. These reports suggest the potentiality of TFs to become a supplementary therapeutic agent in the treatment of microorganism-mediated periodontal diseases.

Antiviral Activity: The human coronavirus, SARS-CoV, was the major causative agent behind the upper respiratory tract illness in humans as well as others animals in the progression of SARS (Severe Acute Respiratory Syndrome) in 2002 [47]. Studies on coronaviruses revealed that all coronaviruses encode a papline like protease (PLP) and a chymotrypsin-like (3CLpro) protease for proteolytic procession during virus maturation, and it has been found that the 3CLpro cleaves at least 11 interdomain sites on the pp1 a & pp 1 ab poly proteins whereas the PLP cleaves only at two sites of pp1 a polyprotein making the 3CL pro a much more attractive drug target for anti-SARS-Cov drug formulation [48]. However, Chen and the co-workers did extensive research and concluded that TF2b (TF3'MG) and TF3 (TF33'DG) along with tannic acid of black tea extract are potential inhibitors of 3CLpro using an HPLC proteolytic assay.

They further compared other tea components like methylxanthine (caffeine and theophylline) and catechins (EGCG, EC, C, ECG, and EGC) with TFs and found that they were unable to inhibit 3CL proactivity at concentrations up to 100 µM [47]. TF2b and TF3 have proven to be more potent inhibitors than TF1 as it lacks the gallate group. Thus, the presence of gallate group at the 3' position of TF2b and TF3 is important for the inhibitory activity against it 3CL pro [47]. In 2019 the human corona virus made an appearance again in the form of Covid-19 and its outbreak is causing a serious, devastating effect on the human population. An *in-silico* study using molecular docking as an important tool Bhatia and her co-workers concluded that the SH6 (Sanguiin-H-6) (hydroxybenzoic acid derivative) is a potent inhibitor with a docking score of -10.3 kcal/mol followed by two derivatives of theaflavin *i.e.*, TF33'DG and TF3MG with equally good docking scores of -10.0 kcal/mol and -9.8 kcal/mol [49].

Clark in his study, reported that theaflavins extracted from black tea can neutralize bovine rotavirus and bovine coronavirus [50]. Bioactive components of Bt like the monomeric catechins, TFs, and flavonol aglycones were reported to be anti-HIV compounds. Molecular docking and molecular simulation revealed TF33'DG to be the most potent inhibitor of gp41 protein [51]. AS a microbicide to prevent HIV sexual transmission by targeting the entry step, vaginal gel formulation based on theaflavin derivatives had been evaluated [52]. Ohba *et al* reported TF2a (TF3MG), TF2b (TF3'MG) and TF3 (TF33'DG) showed broad antiviral activity against three caliciviruses: feline calicivirus, murine norovirus and porcine sapovirus in a study where cytopathic effect-based screening of 2080 selected compounds was done to find the potential antiviral compound against these viruses causing gastroenteritis [53].

Moreover, they conducted a study on the structure-activity relationship of TFs suggesting that the hydroxyl groups of the benzotropolone moiety were important for the anti-calicivirus activity of TFs making them the potential for being an antiviral agent in the prevention of calicivirus infection in animals and humans. Zu *et al.* demonstrated the direct inhibitory effect of TF derivatives on viral particle infectivity of influenza virus by evaluating the gene expression of HA via conducting neuraminidase activity assay, a hemagglutination inhibition assay, a real-time quantitative PCR assay as well as cytopathic effect reduction assay [37].



Anti-mutagenic Activity: Mutagens are any agent be it physical, chemical, or biological that are capable of altering the genetic constituents mainly of a cell by changing the hereditary material *i.e.* DNA thus increasing the frequency of mutations beyond the naturally occurring mutation level. These mutagens causing abnormal mutation often lead to the formation of chronic diseases like cancer. Studies on the effect of theaflavins on mutagenicity have been demonstrated by researchers. Gupta and his co-workers evaluated the antimutagenic effect of two active polyphenols *i.e.* TFs and TRs, in *Salmonella* strains TA97a, TA98, TA100, and TA102 in pre-incubation tests, both with and without S9 activation induced by known bacterial mutagens sodium azide, 4-nitro-o-phenylenediamine, cumene hydroperoxide, 2-aminofluorene, and danthron.

In the study, TFs and TRs exerted a significant antimutagenic effect against known positive compounds in these strains, proving them to be a good antimutagen [54]. In another study conducted by Halder and her co-workers, the anticlastogenic effects of TFs along with TRs were measured by Chromosomal aberration (CA) assay where a significant decrease in CA was observed in all the different concentrations of TF plus benzo [a] pyrene (B[a]P) treated series compared with the control series treated with B[a]P alone [55]. In the SCE (Sister chromatid exchange) assay, TFs and TRs showed positive effects where TFs showed more protective effects, accounting for 13-53% than TRs, which is 8.3-39% against the positive mutagen B[a]P [55]. Again administration of TFs alleviated Cd-induced damage in testis, improvising the sperm characteristics, enhancing the serum testosterone level, and repealing DNA damage [56].

Anticancer Activity: Theaflavin and its galloyl esters of black tea has shown significant inhibitory action against ovarian cancer cell lines OVCAR-3 and A2780/CP70 via apoptotic and antiangiogenic mechanisms. The four main TFs showed anti-proliferative activities against OVCAR-3 and A2780/CP70 cells, where for OVCAR-3 cells, the half-maximal inhibitory concentration (IC_{50}) of TF1 (Theaflavin) was lower than that of the other three and for A2780/CP70 cells, the IC_{50} value of TF1 was highest among all the four major TFs. The cytotoxic effect of theaflavin derivatives on normal ovarian cells like IOSE 364 was measured to test if the derivatives of TF had any adverse effect or not on normal cells. It was found that the normal IOSE

364 cells showed much higher viability when treated with a similar concentration of specific TF derivatives in comparison with the OVCAR-3 and A2780/CP70 cells [57]. A former study conducted by Lu et al. showed that a mixture of TFs inhibits the growth of SV40-transformed WI38 human cells (WI38VA) and Caco-2 colon cancer cells though having a little effect on the normal cells [58]. In human males, prostate gland cancer is the most common type of malignancy as well as the second leading cause of cancer-induced death. In a study, researchers demonstrated the effect of different active components of black tea extract as well as the effect of TFs alone on cellular proliferation and on the mitochondria of the human prostate cancer cell line PC-3 where all the active extracts of black tea and TFs inhibited cellular proliferation, affecting the morphology of PC-3 cells and induced apoptosis, often necrosis of the PC-3 cells [59].

In another study, Gao *et al.* showed the inhibitory effect of Tf33'DG along with EGCG on human lung adenocarcinoma SPCA-1 cells and esophageal carcinoma Eca-109 cells inducing apoptosis enhanced by ascorbic acid [60]. TFs mainly TF33'DG exhibit inhibitory effect on extracellular signal transmission and cell proliferation via inducing cell shrinkage, membrane blebbing and mitochondrial clustering thus triggering apoptosis of cancer cells like mammary epithelial carcinoma cells and leukemia cells [61, 62, 63]. TF33'DG also exerts anti-cancer activity by inhibition of phosphorylation of extracellular signal-regulated kinase-like epidermal growth factor and PDGF receptors of A431 cells and mouse NIH3T3 fibroblast cells [64]. In another study, TFs were reported to inhibit proliferation of A431 and A375 cells by a predicted molecular mechanism where TFs were said to arrest cell cycle via BaX translocation and inducing apoptosis by activating mitochondrial death cascade without affecting the normal human epidermal keratinocyte cells [65].

Anti-metabolic Syndrome: Obesity is the main reason behind metabolic syndrome, causing fatty liver, hypertension, hyperlipidemia consequently leading to diabetes and atherosclerosis. Administration of TFs showed a significant reduction in lipid accumulation, suppressing the synthesis of fatty acid as well as up-regulation of fatty acid oxidation and attenuation of hepatic lipid accumulation *via* stimulating AMP-activated protein kinase in the prevention of obesity [66]. In an investigation, Kobayashi et al. reported the effect of black tea polyphenols on postprandial hypertriacylglycerolemia in rats where the black tea polyphenols inhibited the activity of pancreatic lipase dose-dependently with an IC50 of 0.254 mg/mL. When the activity of the components of black tea was assessed separately on the activity of pancreatic lipase, the TFs with galloyl moieties only were more effective than EGCG, ECG, or a mixture of EGCG and ECG.

The TFs and other components of black tea suppress postprandial hypertriacylglycerolemia via a reduction in the absorption of triacylglycerol by inhibiting pancreatic lipase activity. In contrast with lipase inhibitory activity TFs are also reported to suppress the expression of lipopolysaccharide-induced intercellular adhesion molecules and vascular cell adhesion molecules by blocking the activation of NF-κB and JNK in intestinal epithelial cells [67]. In another report, TFs down-regulates the epidermal growth factor and receptor PI3K/Akt/Sp-1 of the signal transduction pathway, thus suppressing the expression of the key enzyme of lipogenesis *i.e.*, the fatty acid synthase [68]. Vermeer *et al.* proved that Bt theaflavins, particularly TF3MG interfere with intestinal cholesterol absorption [69]. In contrast, Ishikawa documented that Bt theaflavins along with EGCG strongly delay LDL oxidation, thus inhibiting the development and progression of atherosclerosis a consequence of metabolic imbalance [70].

In a study, Matsui *et al.* examined the effects of catechins and TFs against the activity of α-glucosidase, one of the enzymes responsible for the progression of diabetes mellitus. In that report, it was demonstrated that in an immobilized α-glucosidase inhibitory assay system TFs and catechins preferentially inhibited maltase rather than sucrose where the inhibitory effect of TFs on maltase was observed in descending order of potency of TF3MG > TF33'DG > TF3'MG > TF making TF3MG the most potent inhibitor of maltase in comparison with other TF derivatives and catechins. This report suggests TF3MG be a potent supplement against diabetes mellitus as it suppresses glucose production from maltose through the inhibition of α-glucosidase in the gut [71].

Anti-osteoporosis: Osteoporosis is a disease of bones characterized by low bone density as well as structural deterioration of the tissues of bone resulting in fragile bones, increasing the risk of fractures of the spine, hip, and wrist, affecting both men and women equally. Osteoclasts are one kind of bone cell that play a crucial role in maintaining the homeostasis of bones as these are the cells that break down bone tissue as well as are involved in repairing and remodeling bones. These osteoclasts play a major role in the progression of osteoporosis as they produce MMPs *i.e.,* matrix metalloproteinases which are responsible for the degeneration of matrix associated with the bones and cartilage [72].

The MMP-9 is mainly required for the initiation of osteoclastic resorption by removing the collagenous layer from the bone surface before demineralization [73]. Oka *et al.,* in a study, demonstrated that TF33'DG and EGCG suppress the enzymatic activity of MMP-2 & MMP-9 individually, wherein a concentration of 10& 100µm TF33'DG and EGCG were applied individually in cultures of rat osteoclast precursor cells and mature osteoclasts. The results showed that the numbers of multinucleated osteoclasts and actin rings decreased, and the activity of MMP-2 & MMP-9 lowered in TF33'DG and EGCG treated cultures compared to the control set.

The levels of MMP-9mRNA also decreased in the TF33'DG treated sets, thus proving TFs to be more efficient against osteoporosis 74. In another report, TF33'DG has been shown to abrogate bone loss in mice models with osteoporosis by inhibiting SAM-mediated DNA methylation by Dnmt 3a which is the main reason behind the regulation of osteoclastogenesis by epigenetic repression of anti-osteoclastogenic genes [75].

Efficient Mechanisms for the Extraction and Isolation of TFs: The bioactive components are mainly secondary metabolites of plants produced as a by-product of primary metabolism, not affecting the growth & development of plants but confer a selective advantage to the organism as well as elicit pharmacological & toxicological effects in humans and animals. To understand the pharmacological or toxicological effect of a particular bioactive component in humans as well as the animal, the very first step that is needed is to extract and isolate the component in its pure form.

The extraction procedure is crucial, and optimization of extraction condition is a key factor mainly depending on the sample preparation to separate the desired compound from the sample matrix. In contrast, isolation involves the purification of the extracted compound. The TFs are one of the major bioactive constituents of BT and have a greater pharmacological effect on human health, as described earlier. For the extraction and isolation of TFs from the plant matrix, different non-chromatographic and chromatographic techniques are applied, which are discussed in the following-

Extraction Methods: The extraction procedure mainly involves selective separation of one or more compounds from the sample matrix to the solvent system. Various extraction methods are applied to extract different bioactive components of tea, as mentioned earlier. Still, particularly for extraction of TFs, the conventional solvent extraction method is followed, involving direct contact of the solid sample with the liquid solvent. Thus, compounds with similar polarity solubilize into the organic solvent. Many solvents are used to extract theaflavins, and the extraction efficacy depends on the nature of the solvent and extraction time. However, a few efficient extraction techniques for the separation and isolation of TFs are mentioned below.

Ali and the co-workers performed an experiment where 5 g of tea samples from different brands were extracted with 100 ml of methanol in a water bath at a temperature of 60 °C for 10 & 20 min and the extracts were filtered with vacuum filtration assembly. The results showed that the extraction efficacy is maximum in the solvent extraction with methanol for 20 min in comparison with water and methanolic extract of 10 min for TFs. They concluded that extraction time along with temp is a prime factor that affects the concentration of TFs in the final extracts & the longer the extraction time, the higher the yield [3].

In an experiment, Siva and his co-workers for isolation of TFs in their pure form extracted TFs using soxhlet extraction method where 125 g of

powdered tea sample was extracted with ethanol and hexane separately using soxhlet apparatus, and the extraction were carried out for 15 cycles at a temperature of 50 °C, and for further studies, the extract was concentrated using a rotary evaporator at 250 rpm for 72 h at room temp [76].

Kunbo Wang and other co-workers in an experiment extracted TFs via boiling 10 g of tea sample in 150 ml of water followed by filtration and 100 ml of the filtered extract was again extracted with an equal volume of ethyl acetate in a separating funnel where the ethyl acetate phase was collected, filtered through anhydrous sodium sulfate and evaporated to dryness [77].

In another experiment performed by Wang *et al.* for the purification and isolation of TFs, the extraction was done by boiling 5 g of powdered tea in 100 ml of water for 10 min followed by filtration, and the filtrate was again extracted with an equal volume of ethyl acetate for 2 times using separating funnel. The ethyl acetate extract was again mixed with $NaHCO_3$ and shaken for 30 sec, discarding the aqueous layer; the ethyl acetate layer was collected as a pre-treatment before isolation [78].

Yang *et al.* extracted TFs using 70% aqueous ethanol where 20 g of tea were extracted in the ethanolic solution (400 ml 2×) for 40 min in a sonicator (40Hz) at 60 °C and concentrated to 500 ml using a rotary evaporator at 40 °C and then partitioned 2 times with equal volume of chloroform for removal of caffeine. The aqueous layer was collected and subjected to partitioning twice again with an equal volume of ethyl acetate, collecting the ethyl acetate layer, it was evaporated to dryness for HPLC analysis [79].

In another experiment to determine different theaflavins along with methylated ones, Nishimura *et al.* extracted TFs from 50 mg of tea leaf powder three times using 2 ml of 50% ethanolic solution containing 2% of ascorbic acid by shaking at 1500 rpm for 20 min at room temperature [80].

To isolate TFs from black tea Degenhardt used ethyl acetate for preparing the black tea extract where 20 g of black was boiled in 1000 ml of water for 10 min and after cooling the aqueous infusion was extracted with an equal volume of ethyl acetate, which was again evaporated to dryness for preparative chromatographic separation [81]. In a comparative analysis between tea catechins and TFs using HPLC and capillary electrophoresis, the bioactive compounds, *i.e.*, the catechins and TFs were extracted by boiling 0.1 g of tea leaf in 10 ml water and then incubated at 90 °C for 30min including 30 sec of shaking [82].

Isolation Techniques: Where extraction involves the separation of the bioactive components of the plant material from an inert mixture into selective solvent isolation involves the purification of that extracted component. Basically, for the purification of Theaflavins, different chromatographic techniques are applied, involving 1. Thin-layer chromatography (TLC), 2. Column chromatography (CC), 3. High-performance liquid chromatography (HPLC), 4. High-speed counter-current chromatography (HSCCC). The chromatography principle applies the technique of separation and purification of particular compounds from a sample mixture where there are two phases, the mobile phase, which carries the component through a solid structure comprising the stationary phase. A few of the efficient chromatographic techniques applied for the separation and purification of TFs are discussed below:

Thin Layer Chromatography (TLC): The TLC is a separation and identification technique of a mixture of components into individual components by distributing the component mixture into two phases, *i.e.*, the mobile phase and the stationary phase. However, the selection of solvents for constituting the mobile phase is very important as the movement of components depends on the polarity of the solvents. For simultaneous analysis of TFs and catechins, Wang et al performed TLC using different solvent systems for the mobile phase to screen the effect of the solvents on retardation of the major TFs. In the experiment five different solvent systems were used those are: MI-methanol; MII- chloroform-methanol 1:1 (v/v); MIII- methanol–acetic acid 1:1 (v/v); MIV-n-butanol-acetone-water 5:5:3 (v/v); MV- methanol–acetone-formic acid 2:1:1 (v/v) whereas the stationary phase remained same *i.e.* the polyamide plate. The results showed that the MII phase has good resolution separating all the major TFs, and there is a correlation between the concentration of chloroform with the R_f value of TFs, wherewith the

increasing amount of chloroform, the R_f value decreases. The maximum amounts of separation of the four TFs were attained when 40% of chloroform was used with methanol (2:3, v/v, chloroform-methanol, two-fold development) in MI phase [77]. Siva *et al.* followed the similar solvent system *i.e.,* chloroform-methanol in a ratio of 2:3 (v/v) for separation of TFs where 1% w/v solution of the ethanolic black tea extract was used as a crude sample loaded on pre-coated aluminum with silica gel 60 F254 TLC plates constituting the stationary phase and for visualization of the separated bands of TFs the plates were developed by dipping into iron chloride-ethanol reagent [76].

Column Chromatography: Column chromate-graphy preparative technique for purification of components from a sample mixture into individuals by using a column packed with adsorbent depending on the affinity of those components towards the stationary bed (stationary phase) as well as the solvent system (mobile phase). In column chromatography, the stationary phase in use may be of various kinds like silica gel which is the most common; next to it is alumina. Sephadex and cellulose powders are also used as stationary phases in column chromatography. In contrast, the solvent system used in column chromatography is mainly organic solvents depending on the polarity of the components that are to be separated.

For isolation of TFs from the crude ethanolic extract of black tea, Siva and his co-workers performed column chromatography using silica gel (60-120 mesh size) as stationary phase eluted with a mobile phase constituted of acetonitrile solution containing 0.1% formic acid with gradient elution, maintaining flow rate at 1.4mL/min with detection at 260-280 nm [76]. Du *et al.* did a comparative analysis between two different chromatographic methods for isolation of TFs where one of the techniques involved column chromatography using Sephadex LH-20 as a stationary bed and 35% acetone as a mobile phase. 100 mg of crude TF dissolved in 20 ml of water was eluted with 35% of aqueous acetone through a glass column filled with aslurry of Sephadex LH-20 at a flow rate of 0.9 mL/min. The detection was made with a UV-VIS detector [83]. In another experiment by Degenhardt, the lyophilized crude extract of TF was dissolved in ethanol and eluted through Sephadex LH-20 column where first ethanol and 5% acetone in ethanol (v/v) were used as mobile phase to elute all the flavanols, and then 10% acetone in ethanol (v/v) was used for elution of the TFs [81].

Yang *et al.* performed column chromatography using Sephadex LH-20 to isolate and purify TFs from crude extract where 20g of Sephadex LH-20 gel were saturated fully with 200 ml of distilled water and then packed into a column, eluting with 45% of acetone in two folds to equilibrate before loading of sample [79].

High-performance Liquid Chromatography (HPLC): HPLC is another chromatographic separation technique of compounds where crude samples or sample mixtures in a solvent system (mobile phase) are separated and quantified into individual components high pressure through a column comprised of stationary phase. Apart from the two other chromatographic techniques used for the separation and purification of TFs, HPLC is more authentic as it not only purifies all the major TFs but also helps to quantify the amount of them in a sample.

Du *et al.* in a comparative analysis for separation of TFs using various methods, performed HPLC where the experiment was carried out in a BDS column (5 µm, 250 mm × 4.6 mm), and the mobile phase consisted of two solvents- solvent A(acetonitrile) & solvent B (2%,v/v, aqueous acetic acid) following a gradient elution starting with 8% solvent A & 92% solvent B and ending with 69% solvent A & 31% solvent B with a flow rate maintained at 1.4 mL/min [83].

Another experiment for analysis of TFs Cao *et al.* performed HPLC with diode array detection (HPLC-DAD) on a Hypersil C18 column (3 µm, 100 × 4.6 mm) UVD340S diode array detector. The mobile phase in use comprised two different solvents i.e., solvent A (2% acetic acid in acetonitrile) & solvent B (2% acetic acid in high purity water), following gradient elution a flow rate of 1.8 mL/min [84].

For separation of TFs Kumar *et al* did HPLC in a Luna-5 µm Phenyl-Hexyl column (4.6 × 250 nm) with binary gradient using mobile phase A comprising of 9% (v/v) acetonitrile, 2% (v/v) acetic acid with 20 ug/mL EDTA and mobile phase B

comprising of 80% (v/v) acetonitrile2% (v/v) acetic acid with 20 ug/mL EDTA followed by a linear gradient. The detection of the eluted peaks was done at 280 nm, and the flow rate was maintained at 10 mL/min with an injection volume of 10 µL [85].

Degenhardt *et al.* in his experiment, did HPLC-DAD using a Nucleosil RP18 column (5 µm, 510 mm × 4.6 mm) and a diode array detector module 168. Linear gradient elution was followed with two different solvents- solvent A (9% acetonitrile in 2% aqueous acetic acid, v/v/v) & solvent B (80% aqueous acetonitrile, v/v) at a flow rate of 0.8mL/min with peak detection at 280 & 350 nm [81].

Lee also performed HPLC-DAD to purify TFs from crude sample using a C18 cartridge column with a peak detection made at 205 nm following gradient elution where two mobile phases were used- (A) 5% (v/v) acetonitrile with 0.035% (v/v) trifluoroacetic acid & (B) 50% (v/v) acetonitrile containing 0.025% (v/v) trifluoroacetic acid at a flow rate of 1.0mL/min and an injection volume of 10 µL [82].

Using an analytical HPLC method, Nishimura *et al* revealed the optimal mobile phase that can be used for purification of four major TFs as well as two methylated TFs where the mobile phase was a gradient system comprised of two different solvents *i.e.* solvent A (15% acetonitrile/water containing 0.5% acetic acid) and solvent B (80% acetonitrile/water containing 0.5% acetic acid) with a flow rate of 0.2 mL/min and the peak detection was made at 280 nm using a photodiode array UV detector [80].

In an experiment, Wang *et al* did HPLC-DAD for chromatographic separation of TFs using Hypersil C18 column (5 m, 150 mm × 4.6 mm) and SPD-M20A diode array detector following gradient elution with two mobile phases- 2% acetic acid in high pure water (solvent A) and acetonitrile: ethyl acetate (7:1, v/v)(solvent B) at a flow rate of 0.9mL/min with a photodiode array detection (spectra 200-600 nm) [78].

An approach towards isolation and purification of the major TFs from black tea Yang *et al.* performed HPLC along with UV detection using a Nucleosil RP 18 column (3 µm, 100 × 4.6 mm) followed by a linear gradient mobile phase consisting of two solvent systems *i.e.*, 2% aqueous acetic acid (solvent A) & acetonitrile (solvent B) and filtration of all the solvents and samples were done before use through a 0.45 µm filter. The injection volume was 5 µL at a flow rate of 1.2 mL/min followed by peak detection at 280 & 380 nm [79].

High-Speed Counter-current Chromatography (HSCCC): The high-speed counter-current chromatography (HSCCC) is a liquid-liquid chromatographic technique where the partition of the components takes place between two immiscible phases involving a support free liquid stationary phase, a liquid mobile phase, and a centrifugal force field which mainly helps to retain the liquid stationary phase [86]. For isolation and purification of TFs from black tea quickly, HSCCC has been proven more efficient than any other chromatographic technique. However, the various solvents used during HSCCC by the different researchers in approach to isolate& purify TFs from black tea are discussed below.

In a comparative analysis between HSCCC and Sephadex LH20 for separation of TFs the HSCCC experiment was done by Du *et al.* using a two-phase solvent system comprised of hexane-ethyl acetate-methanol-water (1:3:1:6, v/v) where the upper phase was used as stationary phase and the lower aqueous one was the mobile phase with a flow rate maintained at 2.5 mL/min and detection of effluent was monitored with the help of a UV-VIS detector at 380 nm which resulted into a separation efficiency of 12times than that of other techniques [83].

Kumar *et al.* in his experiment, performed HSCCC using the versatile HEMW at a solvent system which is mainly comprised of hexane-ethyl acetate-methanol and water (Hex-EtOAc-MeOH-Water) where hexane and ethyl acetate constitute the upper organic layer of the biphasic mixture and the lower aqueous phase of the biphasic mixture is composed of the rest two *i.e.*, methanol and water. The HSCCC setup consisted of a pump, a multi-coil CCC system, a UV detector, and a recorder followed by three different biphasic solvent systems with different ratios of Hex-EtOAc-MeOH-Water at a flow rate of 2.0 mL/min, 2.5 mL/min, and 2.8 mL/min along with UV detection of the effluents at 280 nm [85].

The solvent system used by Degenhardt for HSCCC to separate TFs were similar to the others *i.e*., hexane-ethyl acetate-methanol-water, whereas the ratio varied from others *i.e.*, 1.5:5:1.5:5 with the less dense layer being the stationary phase and the flow rate was 2.8 mL/min with a UV detection at 280 nm [81].

Wang *et al.* performed HSCCC using the biphasic solvent system composed of hexane-ethyl acetate-methanol-water-acetic acid in a ratio of 1:5:1:5:0.25 at a flow rate of 2.0 mL/min, and the effluent was monitored at 280 nm with the help of a UV-Vis detector [78].

Yang *et al.* followed the similar HEMW at solvent system chosen by the others, *i.e.,* hexane-ethyl acetate-methanol-water though the ratio was 1:3:1:6 where the biphasic solvent was shaken vigorously in a funnel, equilibrated, and the two phases were separated before use. The flow rate was 1.5 mL/min with detection of the effluent at 280 nm using a UV detector [79].

CONCLUSION: Theaflavins being one of the major non-volatile pigments responsible for the characteristic color and astringency of black tea are present at a very low concentration. The various health benefits ascribed to TFs prove its potentiality to become a supplementary therapeutic agent as well as a biochemical modulator for enhancement of the effectiveness of different drugs. Various researchers prove that the absorption of black tea polyphenols in the animal system is higher than that of green tea polyphenols. The polyphenols and the other bioactive components act synergistically in the prevention of chronic diseases. However, to investigate the potentiality of TFs to become a therapeutic agent and enhance the absorption into the animal system using novel strategies, it is important to isolate and purify the TFs in their pure form. To extract and purify TFs in their pure form is a very complex mechanism as the polyphenols are very much susceptible to oxidative degradation, so optimizing the techniques involved in isolation is crucial. Among the various efficient extraction and purification techniques that are discussed in the overview, HSCCC has been proven to be more ideal for purifying the non-volatile pigment as it provides high sample loading, relatively short separation time when compared to the others, and high purity of the isolated fractions thus it is useful for the production of TF fractions in larger quantity. The primary goal of this review was to provide available information about the various health benefits of TFs as well as to unlock the option of choosing the more efficient way of isolation which may result in high purity and high yield of the component within a short period leading towards improvement in bioavailability of the component.

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REFERENCES:

1. Sen G, Sarkar N, Nath M and Maity S: Bioactive components of tea. Arch Food Nutr Sci 2020; 4: 001-009.
2. Chaturvedula VSP and Prakash I: The aroma, taste, color and bioactive constituents of tea. J Med Plants 2011; 5(11): 2110-24.
3. Imran A, Arshad MU, Saeed F, Arshad MS and Imran M: Chemical profiling of some promising black tea brands with special reference to cup quality. Biomed J of Scien & Tech Res 2018; 10(3).
4. Skotnicka M, Chorostowska-Wynimko, Joanna Jankun Jerzy and Skrzypczak-Jankun Ewa: The black tea bioactivity: An overview. Central-European Journal of Immunology 2011; 36: 284-92.
5. Kakuda T: Neuroprotective effects of the green tea components theanine and catechins. Biol Pharm Bull 2002; 25: 1513-18.
6. Ray S and De B: Aectylcholinesterase inhibitory properties of black tea and its polyphenolic components. Int J Pharm Pharm Sci 2012; 4: 334-37.
7. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHAL, Ghafoor K, Norulaini NAN and Omar AKM: Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering 2013; 118: 426-36.
8. Suslick KS and Grinstaff MW: Protein microencapsulation of nonaqueous liquids. Journal of the American Chemical Society 1990; 112(21): 7807-09.
9. Alupului A, Calinescu Ioan and Lavric Vasile: Microwave extraction of active principles from medicinal plants. upb scientific bulletin, series b. Chemistry and Materials Science 2012; 74: 129-42.
10. Xu R, Ye H, Sun Y, Tu Y and Zeng X: Preparation preliminary characterization antioxidant hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia* sinensis). Food Chem Toxicol 2012; 50(7): 2473-80.
11. Herrero Miguel, Cifuentes Alejandro and Ibáñez Elena: Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-

products, algae and microalgae A review. Food Chemistry 2006; 98: 136-48.
12. Sang S: Tea chemistry and processing. Encyclopedia of Food and Health 2016; 268-72.
13. Mukhtar H and Ahmad N: Tea poly phenols: prevention of cancer and optimizing health. Am J Clin Nutr 2000; 71: 1698-02.
14. Hara Y, Luo SJ, Wickremashinghe RL and Yamanishi T: Botany (of tea). Food Rev Int 1995; 11: 371-74.
15. Liang Y, Lu J, Zhang L, Wu S and Wu Y: Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. Food Chem 2003; 80: 283-90.
16. Hara Y, Luo SJ, Wickremashinghe RL and Yamanishi T: Processing tea. Food Rev Int 1995; 11: 409-34.
17. Balentine DA: Introduction tea and health. Crit Rev Food Sci Nutr 1997; 8: 691-69.
18. Collier PD, Bryce T, Mallows R, Thomas PE, Frost DJ, Korver O and Wilkins C: The theaflavins of black tea. Tetrahedron 1973; 29: 125-42.
19. Engelhardt UH: Chemistry of tea. Elsevier 2010.
20. Miller NJ, Castelluccio C, Tijburg L and Rice EC: The antioxidant properties of theaflavins and their gallate esters--radical scavengers or metal chelators. FEBS Lett 1996; 19: 40-44.
21. Van Acker S, van Balen G, van den Berg D, Bast A and Van der Vijgh W: Influence of iron chelation on the antioxidant activity of flavonoids. Biochem Pharmacol 1998; 56: 935-43.
22. Sharma Pallavi, Jha Ambuj, Dubey Rama and Pessarakli Mohammad: Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012; 26.
23. Leenen R, Roodenburg AJ and Tijburg LB: A single dose of tea with or without milk increases plasma antioxidant activity in humans. Eur J Clin Nutr 2000; 54: 87-92.
24. Alexander Y, Yakov Y and Boris N: Determination of antioxidant activity in tea extracts and their total antioxidant content. American Journal of Biomedical Sciences 2011; 3.
25. Vasundhara S, Honnaiah VK and Jagan Mohan Rao L: Influence of milk and sugar on antioxidant potential of black tea. Research International 2008; 41(2): 124-29.
26. Eric WCC, Eu YS, Pei PT and Yon PL: Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia* sinensis. Pharmacognosy Res 2011; 3: 266-72.
27. Hong L, Xiaofeng M, Chuan L, Shengmin S and Christopher P: Glucuronides of tea catechins: enzymology of biosynthesis and biological activities. Drug Metab Dispos 2003; 31: 452-61.
28. Slobodan VJ and Micheal GS: Antioxidants in nutrition. Ann New York Acad Sci 2000; 899: 326-34.
29. Kyoji Y, Isao T, Mitsuaki S, Itaro O and Yukihiko H: Effects of long-term dietary supplement of tea poly phenols on lipid peroxide levels in rats. Age 1994; 17: 79-85.
30. Ya LS, Lai KL, Yu H and Zhen YC: Stability of tea theaflavins and catechins. Food Chem 2003; 83: 189-95.
31. Sheila AW, Douglas AB and Balz F: Antioxidants in tea: critical reviews in food science and nutrition. Tea and Health 1997; 37(8): 705-18.
32. Łuczaj W and Skrzydlewska E: Antioxidative properties of black tea. Prev Med 2005; 40(6): 910-18.
33. Samir M, Tanmay SN, Koushik B, Adinpunya M and Ghosh BC: Protective effect of tea against copper (Cu) toxicity in erythrocyte. International Journal of Tea Science 2013; 12-25.
34. James WD, Michael NC, Jacek O and Nikolai K: The chemistry of low molecular weight black tea poly phenols. Nat Prod Rep 2010; 27: 417-62.
35. Arent SM, Senso M and Golem DL: The effects of theaflavin-enriched black tea extract on muscle soreness, oxidative stress, inflammation and endocrine responses to acute anaerobic interval training: a randomized, double-blind, crossover study. J Int Soc Sport Nutr 2010; 7(1): 11.
36. Huimei C, Yangyang D, Yeyun L, Daxiang L and Chuanyi P: Physiological and cellular responses to fl uoride stree in tea (*Camellia* sinensis) leaves. Acta Physiologiae Plantrum 2016; 38: 144.
37. Zu M, Yang Fm and Zhou W: *In-vitro* anti-influenza virus and anti-inflammatory activities of theaflavin derivatives. Antiviral Res 2012; 94(3): 217-24.
38. Gosslau A, Jao DLE and Huang MT: Effects of the black tea polyphenol theaflavin-2 on apoptotic and inflammatory pathways *in-vitro* and *in-vivo*. Mol Nutr Food Res 2011; 55(2): 198-08.
39. Hosokawa Y, Hosokawa I, Ozaki K, Nakanishi T, Nakae H and Matsuo T: Tea poly phenols inhibit IL-6 production in tumor necrosis factor super family 14-stimulated human gingival fibroblasts. Mol Nutr Food Res 2010; 54: S2.
40. Hosokawa Y, Hosokawa I and Shindo S: Black tea polyphenol inhibits CXCL10 production in oncostatin M-stimulated human gingival fibroblasts. Int Immunopharmacol 2011; 11(6): 670-74.
41. Cai F, Li CR and Wu JL: Theaflavin ameliorates cerebral ischemia-reperfusion injury in rats through its anti-inflammatory effect and modulation of STAT-1. Mediat Inflamm 2006; 5: 30490.
42. Anandhan A, Essa MM and Manivasagam T: Therapeutic attenuation of neuroinflammation and apoptosis by black tea theaflavin in chronic MPTP/probenecid model of Parkinson's disease. Neurotox Res 2013; 23(2): 166-73.
43. Slots J: Periodontology: Past, present, perspectives. Periodontol 2013; 62: 7-19.
44. Zhou J and Windsor LJ: Porphyromonas gingivalis affects host collagen degradation by affecting expression, activation and inhibition of matrix metalloproteinases. J Periodontal Res 2006; 41: 47-54.
45. Matsushita K, Imamura T, Tomikawa M, Tancharoen S, Tatsuyama S and Maruyama I: DX-9065a inhibits pro inflammatory events induced by gingipains and factor XA. J Periodontal Res 2006; 41: 148-56.
46. Kong L, Qi X, Huang S, Chen S, Wu Y and Zhao L: Theaflavins inhibit pathogenic properties of P. gingivalis and MMPs production in P. gingivalis-stimulated human gingival fibroblasts. Arch Oral Biol 2015; 60: 12-22.
47. Chen C-N, Lin CP and Huang K-K: Inhibition of SARS-CoV 3C-like protease activity by theaflavin-3, 3′digallate' (TF3). Evid Based Complement Alternat Med 2005; 2(2): 209-15.
48. Hegyi A, Friebe A, Gorbalenya AE and Ziebuhr J: Mutational analysis of the active centre of corona virus 3C-like proteases. J Gen Virol 2002; 83: 581-93.
49. Bhatia S, Giri S, Lal AF and Singh S: Identification of potential inhibitors of dietary polyphenols for SARS-CoV-2 M protease: An *in-silico* study. Tropi Pub Heal 2020; 1(1): 21-29.
50. Clark KJ, Grant PG, Sarr AB, Belakere JR, Swaggerty CL and Phillips TD: An *in-vitro* study of theaflavins extracted from black tea to neutralize bovine rotavirus and bovine corona virus infections. Vet Microbiol 1998; 63: 147-57.
51. Mostashari RT, Saghaei L and Fassihi A: Gp41 inhibitory activity prediction of theaflavin derivatives using

ligand/structure-based virtual screening approaches. Comput Biol Chem 2019; 78: 119-26.
52. Yang J, Li L and Tan S: A natural theaflavins preparation inhibits HIV-1 infection by targeting the entry step: potential applications for preventing HIV-1 infection. Fitoterapia 2012; 83(2): 348-55.
53. Ohba M, Oka T, Ando T, Arahata S, Ikegaya A, Takagi H, Ogo N, Zhu C, Owada K and Kawamori F: Antiviral effect of theaflavins against caliciviruses. J Antibiot Tokyo 2017; 70: 443-47.
54. Gupta S, Chaudhuri T and Seth P: Antimutagenic effects of black tea (World Blend) and its two active poly phenols theaflavins and thearubigins in *salmonella* assays. Phytother Res 2002; 16: 655-61.
55. Halder B, Pramanick S and Mukhopadhyay S: Inhibition of benzo [a] pyrene induced mutagenicity and genotoxicity by black tea polyphenols theaflavins and thearubigins in multiple test systems. Food Chem Toxicol 2005; 43(4):m 591-97.
56. Wang W, Sun Y and Liu J: Protective effect of theaflavins on cadmium-induced testicular toxicity in male rats. Food Chem Toxicol 2012; 50(9): 3243-50.
57. Gao Y, Rankin GO, Tu Y and Chen YC: Inhibitory effects of the four main theaflavin derivatives found in black tea on ovarian cancer cells. Anticancer Res 2016; 36: 643-51.
58. Lu J, Ho CT, Ghai G and Chen KY: Differential effects of theaflavin monogallates on cell growth, apoptosis and COX-2 gene expression in cancerous versus normal cells. Cancer Res 2000; 60(22): 6465-71.
59. Sun S, Pan S, Miao A, Ling C, Pang S, Tang J, Chen D and Zhao C: Active extracts of black tea (*Camellia* Sinensis) induce apoptosis of PC-3 prostate cancer cells via mitochondrial dysfunction. Oncol Rep 2013; 30: 763-72.
60. Gao Y, Li W and Jia L: Enhancement of (-)-epigallocatechin-3-gallate and theaflavin-3, 3′-digallate induced apoptosis by ascorbic acid in human lung adenocarcinoma SPC-A-1 cells and esophageal carcinoma Eca-109 cells via MAPK pathways. Biochem Biophys Res Commun 2013; 438(2): 370-74.
61. Koňariková K, Ježovičová M and Keresteš J: Anticancer effect of black tea extract in human cancer cell lines. Springer Plus 2015; 4: 127.
62. Lahiry L, Saha B and Chakraborty J: Contribution of p53-mediated Bax transactivation in theaflavin-induced mammary epithelial carcinoma cell apoptosis. Apoptosis 2008; 13(6): 771-81.
63. Kundu T, Dey S and Roy M: Induction of apoptosis in human leukemia cells by black tea and its polyphenol theaflavin. Cancer Lett 2005; 230(1): 111-21.
64. Liang Y-C, Chen Y-C and Lin Y-L: Suppression of extracellular signals and cell proliferation by the black tea polyphenol, theaflavin-3, 3′-digallates. Carcinogenesis. 1999; 20(4): 733-36.
65. Halder B, Pramanick S and Mukhopadhyay S: Anticlastogenic effects of black tea poly phenols theaflavins and thearubigins in human lymphocytes *in-vitro*. Toxicol *In-vitro* 2006; 20(5): 608-13.
66. Lin C-L, Huang H-C and Lin J-K: Theaflavins attenuate hepatic lipid accumulation through activating AMPK in human HepG2 cells. J Lipid Res 2007; 48(11): 2334-43.
67. Song Y-A, Park Y-L and Yoon S-H: Black tea poly phenol theaflavin suppresses LPS-induced ICAM-1 and VCAM-1 expression via blockage of NF-κB and JNK activation in intestinal epithelial cells. Inflamm Res 2011; 60(5): 493-500.
68. Yeh C-W, Chen W-J and Chiang C-T: Suppression of fatty acid synthase in MCF-7 breast cancer cells by tea and tea polyphenols: a possible mechanism for their hypolipidemic effects. Pharmacogenom J 2003; 3(5): 267-76.
69. Vermeer MA, Mulder TP and Molhuizen HO: Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. J Agric Food Chem 2008; 56: 12031-36.
70. Ishikawa T, Suzukawa M annd Ito T: Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. Am J Clin Nutr 1997; 66: 261-66.
71. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata Y, Tanaka K and Matsumoto K: Alpha-Glucosidase inhibitory profile of catechins and theaflavins. J Agric Food Chem 2007; 55: 99-105.
72. Tezuka K, Nemoto K, Tezuka Y, Sato T, Ikeda Y, Kobori M, Kawashima H, Eguchi H, Hakeda Y and Kumegawa M: Identification of matrix metalloproteinase 9 in rabbit osteoclasts. J Biol Chem 1994; 269: 15006-09.
73. Logar DB, Komadina R, Prezelj J, Ostanek B, Trost Z and Marc J: Expression of bone resorption genes in osteoarthritis and in osteoporosis. J Bone Miner Metab 2007; 25: 219-25.
74. Oka Y, Iwai S and Amano H: Tea poly phenols inhibit rat osteoclast formation and differentiation. J Pharmacol Sci 2012; 118(1): 55-64.
75. Nishikawa K, Iwamoto Y, Kobayashi Y, Katsuoka F, Kawaguchi S, Tsujita T, Nakamura T, Kato S, Yamamoto M and Takayanagi H: DNA methyltransferase 3a regulates osteoclast differentiation by coupling to an S-adenosylmethionine-producing metabolic pathway. Nat Med 2015; 21: 281-87.
76. Siva GV, Kirubaasnanthan G and Alagarasan C: Extraction of theaflavin from black tea and its bioconjugation with chitin. Int J Curr Biotechnol 2017; 5(7): 1-6.
77. Wang K, Liu Z, Huang J, Fu D, Liu F, Gong Y and Wu X: TLC separation of catechins and theaflavins on polyamide plates. Journal of Planar Chromatography 2009; 22: 97-100.
78. Wang K, Liu Z, Huang JA, Dong X, Song L and Pan Y: Preparative isolation and purification of theaflavins and catechins by highspeed counter-current chromatography. Journal of Chromatography 2008; 86: 282-86.
79. Yang C, Li D and Wan X: Combination of HSCCC and Sephadex LH-20 methods. An approach to isolation and purification of the main individual theaflavins from black tea. Journal of Chromatography B 2003; 861: 140-44.
80. Nishimura M, Ishiyama K, Watanabe A, Kawano S, Miyase T and Sano M: Determination of theaflavins including methylated theaflavins in black tea leaves by solid-phase extraction and hplc analysis. J Agric Food Chem 2007; 55: 7252-57.
81. Degenhardt A, Engelhardt UH, Wendt AS and Winterhalter P: Isolation of black tea pigments using high-speed counter-current chromatography and studies on properties of black tea polymers. Journal of Agricultural and Food Chemistry 2000; 48: 5200-05.
82. Lee B-L and Ong C-N: Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis. J Chromatogr A 2000; 881: 439-47.
83. Qizhen Du, Heyuan Jiang and Yoichiro Ito: Separation of theaflavins of black tea. high-speed counter-current chromatography vs. sephadex lh-20 gel column

chromatography. J Liq Chrom & Rel Technol 2001; 24(15): 2363-69.
84. Cao X, Lewis JR and Ito Y: Application of high-speed counter-current chromatography to the separation of black tea theaflavins. J Liq Chromatogr Relat Technol 2004; 27(12): 1893-02.
85. Savitri Kumar N, Nimal Punyasiri PA and Wijekoon WAMB: The hexane-ethyl acetate-methanol-water system for the separation of theaflavins from black tea (*Camellia* sinensis) using high-speed counter-current chromatography. Ceylon J Sci 2016; 45(2): 79-86.
86. Sethi N, Anand A, Sharma A, Chandrul K, Jain G and Srinivasa Kona: High speed counter current chromatography-a support free lc technique. Journal of Pharmacy and Bio Allied Sciences 2009; 1(1): 14-22.

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