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Review: anti-influenza viral effects of camellia tea

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Abstract: Influenza virus cause 3–5 million severe medical cases, including nearly 500,000 respiratory deaths globally annually. Even with vaccines and antiviral drugs, it always manages to re-emerge. In traditional medicine, *Camellia sinensis* tea is used as a remedy and prevention against Influenza. This article researched previous literature rigorously to analyse the anti-influenza viral effects of camellia tea, experimental designs, gaps in previous work and limitations of employed approaches. In literature, epigallocatechin gallate (EGCG) and theaflavin digallate (TF3) in camellia tea had shown prominent anti-viral properties against influenza. Clinical approach, in-vitro and social survey were commonly used approaches while majority were clinical approaches. Surveys were least used. The accuracy of the in-vitro was highest, while social surveys being least accurate. We further noticed that the exact mechanisms, pharmacokinetics, pharmacodynamics and toxicology of these compounds as independent or as mixtures in the real human body are not yet fully known.

Keywords: influenza; camellia tea; anti-viral; biochemical; clinical.

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1 Introduction

Even though fermentation had been the oldest form of food processing in human history which runs even beyond 7000 BC where Chinese used to produce an alcoholic beverage called Kui, made with rice, honey, grapefruits, and hawthorn in their legendary culinary arts (Prajapati and Nair, 2008) and the concept of putrefaction had been a closer phenomenon related with the life cycle of every living being, the spotlight of medical concern had not been focused on these both scenario until the second half of the nineteenth century. With the discovery of the germ theory of disease, by the French scientist Louis Pasteur, in 1876, the dawn of a new discipline called 'bacteriology' was begun. But the question 'Are all these infectious tiny matters really bacteria?' was arisen as the discipline got explored by researchers with greater curiosity, the development of the latest microscopic techniques and technological advancement, as well as the discovery of these unique particles called 'tobacco mosaic virus' in 1892, challenging the dogma of studying them under the 'bacteriology' (Artenstein, 2012). As more variations of those unique particles which were quite different from bacteria but infectious as bacteria started to step out of shadows due to the expansion of human intelligence and intuition over the next five decades, scientists started to treat the subject not as a mere branch of pathology but as a biological science, akin to bacteriology (Luria, 1953). As a result, 'virology' was branched out as an independent discipline in around 1950; the late twentieth century (Van Helvoort, 1994). So far, certain studies have estimated that a minimum of 320,000 mammalian viruses exists on Earth (Anthony et al., 2013) and 219 virus species are known to be able to infect humans (Woolhouse et al., 2012). Regardless of the size of the virus, in the global history, smallpox had killed 500 million and Spanish flu had killed 100 million. Hong Kong flu had killed at least 1 million according to estimations. Nearly 22,000 people die by dengue every year and WHO estimated annual mortality burden of influenza is 250,000–500,000 all around the globe

(Paget et al., 2019). Death poll of 'severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)' is still adding up even at the moment. When the impact of these tiny particles were severely high beyond their spectrum and it had erupted immeasurably immense misery upon human species in medical context, human in return had been working on curing, preventing and mitigating the effects of those tiny particles for decades within the realms of virology, medicine, immunology, epidemiology as well as in pharmaceutical sciences, etc. Even before these nano scale particles were identified or named as viruses, human had used the natural herbs to cure and strengthen the human immunity in the battle against those viruses knowingly or unknowingly. Later, the effective chemicals contained in herbs and recipes of those traditional treatments were discovered and isolated with expanding modern pharmacological concepts (Kong et al., 2009). In this context, *Camellia sinensis* or camellia tea had played a major role for centuries when it comes to strengthening the immune system and as a cure for many diseases in traditional medicines in many cultures (Zhen, 2002). *Camellia sinensis* is a flowering plant in the family 'Theaceae', which leaves and leaf buds are used to produce Chinese tea. Based on the processing and different oxidation levels obtained, mainly four varieties had been produced as white tea, green tea, oolong and black tea. The different chemicals that had been amplified in different varieties had been effective on treating different medical conditions and illnesses (Namita et al., 2012). The effect of such chemical compounds existing in *camellia* tea had been discussed all over the literature for decades when it comes to fighting against influenza virus in history.

This study, had tried to integrate and summarise the literature on properties of the virus, the active compounds of camellia tea with anti influenza function, epidemiological and pathological experimental approaches used to assess those properties throughout the literature in extensive but organised manner.

2 Method

Previous literature and cases studies that had been published in research databases such as CORE, ScienceOpen, Directory of Open Access Journals, Public Library of Science, Elsevier, BioMed Central, PubMed, etc. related to impacts of camellia tea on influenza, as well as other medical conditions, were collected and thoroughly researched.

The information were classified under:

- taxonimic significance in ededemiology
- molecular biological structure and viral progeny
- pathology, treatment approaches and natural compounds role
- active compounds of camellia tea (*Camellia sinensis*)
- experimental designs and methodologies employed to assess the effects of those active compounds on influenza infection.

The discovered literature facts had been descriptively and critically analysed to justify the effects, assess the gaps and limitations in previous research efforts and suggest improvements for a proper understanding of mechanisms of camellia tea in immune responses in infected cells.

3 Results and discussion

3.1 Taxonimic significance in ededemiology

As for the taxonomic significance in epidemiological dynamics, influenza is a virus that belongs to family Orthomyxoviridae. The Orthomyxoviridae appeared to be consisting of six types/genera of influenza as *alphainfluenzavirus* (A), *betainfluenzavirus* (B), *gammainfluenzavirus* (C), *thogotovirus*, *isavirus* and *deltainfluenzavirus* (D) (Szewczyk et al., 2014). However, only influenza A, B, and C were found to be infectious to humans (Oxford and Hockley, 1987) in literature. Type D and *thogotovirus* were found to be contagious to other vertebrates such as cattle, camels, and sheep mainly (Strauss and Strauss, 2008) while *isavirus* was recorded to be infecting fish such as sea trout (*Salmo trutta*), rainbow trout and Atlantic herring (Leong, 2008). But clinically, influenza A and B are important viruses to humans since A virus is the classic pandemic virus with a blood trail in human epidemiology history (Oxford and Hockley, 1987).

As for the transmission significance, most recorded influenza strains were type A (subtypes HINl, H2N2, H3N2), type B and type C (Lennette et al., 2012). Many transmission sources had been identified such as human-to-human transmission and animal to human. Even though the most common way is human-to-human transmission, occasionally it had been transmitted from non-human such as swine to humans (*British Medical Journal*, 1973). Usually, Avian influenza had been using swine as an intermediate host to mutate and to be introduced to the human population quite often in history (Scholtissek, 1994).

3.2 Molecular biological structure and viral progeny

Orthomyxoviridae are enveloped negative-single strand RNA (ssRNA) viruses. The genome of influenza virus was consisting of 7 to 8 major gene segments (McCauley and Mahy, 1983). A and B, eight genes and C, seven segments (Taubenberger and Morens, 2008). Those segments were encoding:

Segment 1 basic polymerase protein 2 (PB2)

Segment 2 basic polymerase protein 1 (PB1)

Segment 3 acidic polymerase protein (PA)

Segment 4 hemagglutinin (HA)

Segment 5 nucleoprotein (NP)

Segment 6 neuraminidase (NA)

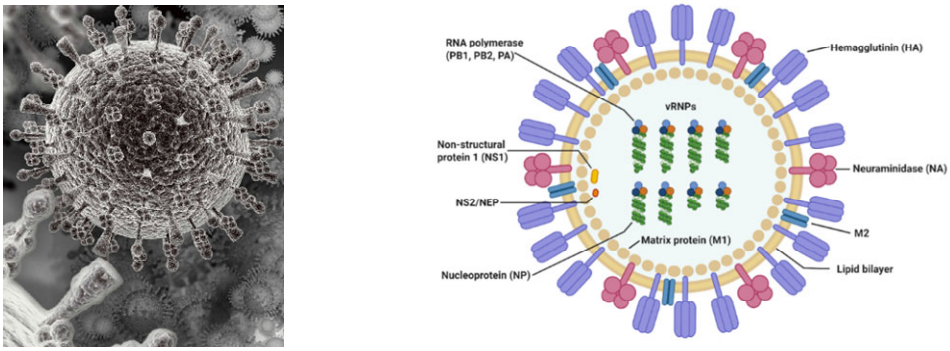
Segment 7 matrix proteins (M1 and M2)

Segment 8 non-structural proteins (NS1 and NS2).

This envelope was derived from the host's cell membrane and composed as a lipid bilayer (Cheung and Poon, 2007). Capsid was consisting of compositionally different antigenic glycoproteins called hemagglutinin (HA) and neuraminidase (NA) (Clancy, 2008). 18, HA types and 11, NA types had been discovered so far in influenza A and different combinations of these had given rise to 198 subvarients of virus (Centers for Disease Control and Prevention, 2017). However from above 198, just three subtypes of

hemagglutinin (H1, H2 and H3) and 2 of neuraminidase (N1 and N2) had been discovered to be responsible for massive epidemics in human history (Bouvier and Palese, 2008).

Figure 1 (a) Scanning electron microscopy – avian flu virus (b) Labelled structure diagram of influenza A virus (see online version for colours)



(a) (b)

Source: Cheung and Poon (2007), [a]https://www.pinterest.com/pin/164311086381294364/?nic_v2=1a2QBTXRC, photo credit to Cahayla and [b]Jung and Lee (2020)

In viral projeny and infection, rod-shaped HA spikes are bound to receptors on the surfaces of epithelial cells in the nose, throat, and lungs of mammals and mushroom shaped NA proteins hydrolyses, sialic acid groups of glycoproteins and destroy receptors in order to release the viral progeny (Cheung and Poon, 2007). M2 channel proteins in the envelope regulate pH across the viral membrane during the process of cell entry. The viral core is acidified with the involvement of M2 ion channels and viral RNA (vRNA), accessory proteins, and RNA-dependent RNA polymerase are released from the dissembled core into the cytoplasm. It also equilibrates pH across the trans-Golgi membrane of infected cells at the viral maturation phase (Pielak and Chou, 2011). Next complementary positive-sense vRNA are being transcribed by the RNA-dependent RNA polymerase in the cell nucleus of the host. Then, complementary vRNA are entered into the cytoplasm and is translated. Newly synthesised viral proteins are either moved the cell surface to form capsids (HA and NA) over the host cell membrane or transported to bind with replicated vRNA in the new viral genome. Meanwhile, some of those virus proteins inhibit the essential mRNA synthesis of host cells required for other purposes

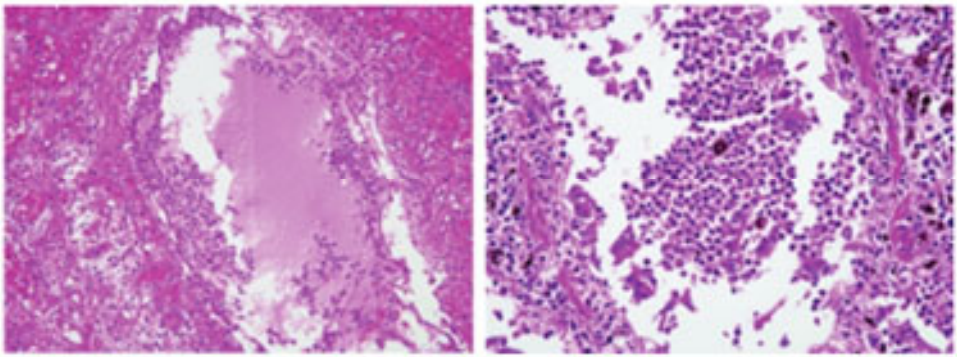
M1 protein layer beneath the viral envelope attaches with ribonucleoproteins (RNP) in the budding process of virus (Hilsch et al., 2014). Finally, newly synthesised negative-sense vRNAs, RNA-dependent RNA polymerase, and other viral proteins are moved into a protrusion on the host cell membrane and budded into new viral particles (Lamb and Choppin, 1983).

3.3 Pathology, treatment approaches and natural compounds role

In pathological context, influenza viruses infect the columnar epithelium lining the respiratory tract. It is capable of targeting, both the upper and lower respiratory tract.

Under fatal conditions, it could lead to complications such as primary viral pneumonia and secondary bacterial pneumonia (Taubenberger and Morens, 2008). On rare occasions, other medical complications such as myositis, myocarditis, and encephalitis also could be occurred (Lennette et al., 2012).

Figure 2 H&E-stained sections of the lung from influenza subjects in 1918, showing necrotising bronchiolitis, epithelial layer had been desquamating, and necrotic epithelial cells are visible in the lumen, (a) 40 × (b) 200 × (see online version for colours)



(a) (b)

Laboratory diagnosis is mainly based on the isolation and identification of virus, or variation of specific antibody titer between serum specimen collected at onset of disease (acute-phase) and two to three weeks later collected specimens (convalescent-phase). When samples are tested by hemagglutination inhibition, neutralisation, enzyme immunoassay, or complement fixation, the rise of titer could be a positive indication of an infection (Palmer, 1975).

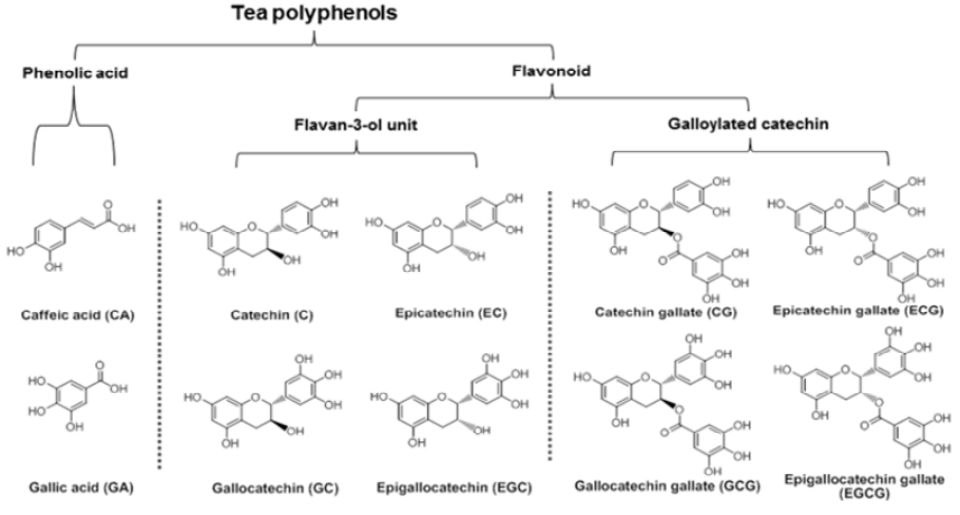
In treating influenza, many treatment regimens such as M2 protein ion channel inhibitors (amantadine and rimantadine), NA protein inhibitors (oseltamivir and zanamivir), laninamivir, favipiravir and peramivir, hemagglutinin inhibitors (EB peptide, peptide NDFRSKT, fludase, a neuraminidase mimics), anti-inflammatory drugs, statins, sphingosine mimics, nuclear factor-kappaB inhibitors, antimicrobial peptides, and proteins, defensins, cathelicidins: LL-37, collectins, as well as short interfering RNA had been used in literature to treat the symptoms, side effects of virus as well as to disturb the function and lifecycle of the virus, based on the specificity of the situation (Barik, 2012).

In the traditional medicinal context active ingredients such as polyphenols, flavonoids, saponins, glucosides, and alkaloids extracted from *Geranium sanguineum L.*, *Cydonia oblonga Mill.*, *Citrus junos*, Tanaka (Rutaceae), Bupleurum Chinense DC, etc. had been widely used in literature (Wang et al., 2006). Applications of green tea as an antiviral agent had been tried as a part of the above traditional biochemistry approach in many cases and had been proven promising in many ways as a part of the solution (Matsumoto et al., 2011). It is anti-inflammatory and antimicrobial peptides properties that active ingredients contained in tea are used in treatment regiments.

3.4 Active compounds of camellia tea (Camellia sinensis)

Green tea, as well as black tea, was consisting of many polyphenolic compounds with antioxidant, anti-inflamatory, anticancer, anti-hypercholesterolemic as well as anti-obesity properties (Clement, 2009). Phenolic acids and flavonoids are two major categories of such chemicals.

Figure 3 Chemical structures and classifications of tested tea polyphenols in green tea



Source: Du et al. (2012)

Phenolic compounds are compounds that have one or more hydroxyl groups attached directly to an aromatic ring. With carboxylic groups attached to these phenolic compounds, they become phenolic acids. When these phenolic compounds are C_{15} compounds that have the structure of C_6-C_3-C_6 they are called flavonoids (Vermerris and Nicholson, 2007).

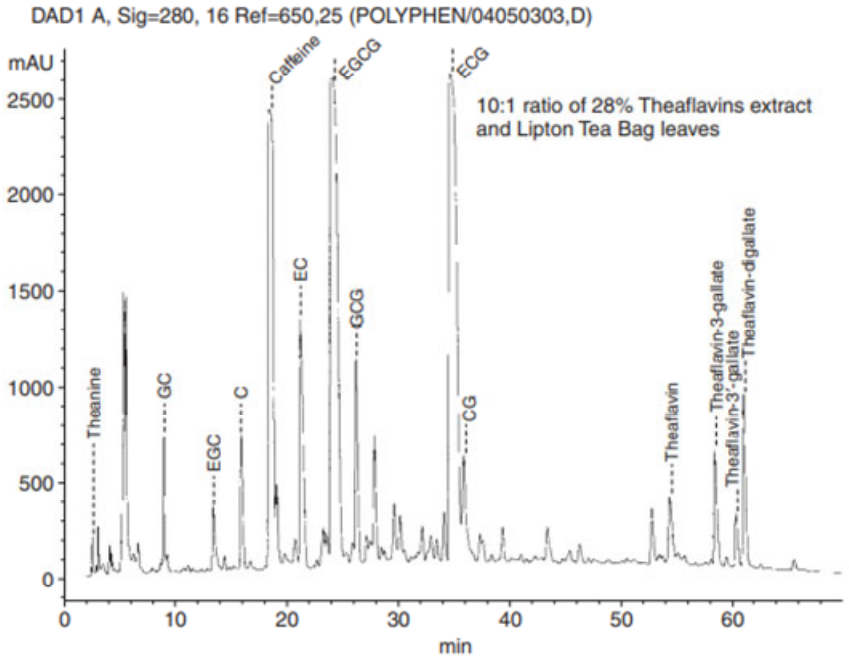
All of those chemical compounds possess various biochemical and therapeutic features. Even though, when it comes to fighting against viruses especially preventing infection of influenza, epigallocatechin gallate (EGCG) and theaflavin digallate (TF3) were two major players in green tea and black tea. EGCG is a galloylated catechin while TF3 is Flavan-3-ol which both belong to the flavonoids subcategory.

Experimental designs and methodologies employed to assess the effects of those active compounds on influenza infection.

When considering about previous literature, three main experimental designs to assess the affectivity of camellia tea (black or green) against influenza infections were discovered. They were:

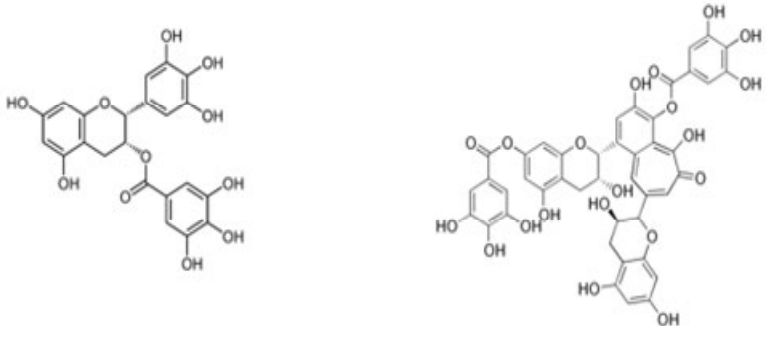
- clinical trials and diagnosis
- in-vitro analysis
- metadata/social survey analysis.

Figure 4 High performance liquid chromatography profile of main chemical constituents in green tea and black teas



Source: Cooper et al. (2005)

Figure 5 Molecular structures of (a) (–)EGCG and (b) TF3



(a) (b)

Source: Du et al. (2012)

In *clinical trials and diagnosis*, a test population had been divided into two groups. The first group had been tasked with gargling green tea and the second with a placebo such as water separately for ninety days in the flu season under controlled conditions. A number of administrations per day, sampling techniques as well as sampling frame/parameters had been pre-determined in the research designing. The primary outcomes had been quantified by influenza infection cases confirmed with the presence of viral antigens via immunochromatographic assay or clinical symptoms such as sore throat, cough, fever,

headache and myalgia (Toyoizumi et al., 2013). Based on the nature of the population and pre-determined frames such as age groups such as high school students (Ide et al., 2014), healthy adults (Yamada et al., 2007) as well as elderly nursing home residents (Yamada et al., 2006) had been used in many previous works. Some research designs had administered catechin/theanine capsules and placebo instead of gargling considering the high risk of exposure factor as well (Matsumoto et al., 2011). In all methods, preliminary information such as weight, behaviour patterns, lifestyle had been collected via surveys, and verification and quantification had been done clinically regardless of method of administration. This type of researches had been done not only in eastern regions such as Japan (Toyoizumi et al., 2013) but also in western regions such as Florida, USA (Rowe et al., 2007) and by comparison of two such situations, the contribution of genetic differences also could be analysed.

In *in-vitro analysis*, tea extraction had been prepared by heating tea leaves with different solvents appropriately and centrifuging. Predefined influenza virus serotypes had been grown in appropriate growth mediums and stored in Allantoic fluids under minus temperatures. Madin-Darby canine kidney (MDCK) cells are cultured separately. Then, virus had been introduced to MDCK cells after being exposed to tea extraction (Nakayama et al., 1990). Finally, plaque assays were performed (Tobita, 1975). Certain research designs had been extracted individual chemical components such as EGCG, EGC, TF3, etc. via chromatographic techniques (Cooper et al., 2005) and tested the effect separately on virus (Du et al., 2012). Generally, 'plaque formation assay' had been used at a basic level to see the effects (Nakayama et al., 1990). In advanced research designs, virus growth inhibition assays (Song et al., 2005), hemagglutination inhibition assays (Nakayama et al., 1993), quantitative RT-PCR analysis (Kim et al., 2013), neuraminidase inhibition assays (Song et al., 2005), as well as florescence microscopic techniques (Müller and Downard, 2015), etc., had been done to test the advance mechanism of catechins in tea that reduces influenza infection. On rare occasions $\gamma\delta T$ cell antigen stimulations also had been run to assess immunological responses (Rowe et al., 2007).

3.4.1 Plaque assays

In the process, an extract of (approximately 200 pfu) pre-determined virus had been inoculated in a confluent monolayer of MDCK cells in medium plates followed by an addition of serially diluted tea extract. The specimens had been exposed to virus for 60 minutes, washed with pre-warmed minimum essential medium (MEM) twice and replaced into Dulbecco's modified eagle medium (DMEM). After the specimen being incubated for four days at 37°C in 5% CO_2 environment, the MDCK monolayers had been fixed with formalin and washed out with flowing tap water. The fixed specimen had been stained with 0.038% methylene blue or 1% crystal violet solution and plaques are counted manually with microscopic techniques (Nakayama et al., 1990). Percentage of plaque inhibition relative to controls, half-maximal effective concentration, dose-response curves, etc. were calculated statistically.

3.4.2 Virus growth inhibition assays

MDCK cells cultured in cell plate as confluent monolayers were infected with predefined influenza strains at predetermined multiplicity of infection (MOI) value, after being washed by phosphate buffered saline (PBS). Then, cell plates had been stored in a shaker

for 45 min at room temperature under compounds-free conditions allowing virus adsorption. The specimen was replaced into MEM with different catechins concentrations. Multiple plaque assays had been conducted on specimens at 8, 24, 36 hour time intervals to estimate virus yields (Song et al., 2005).

3.4.3 Hemagglutination inhibition assays

A virus suspension mixed with an equal volume of tea extracts had remained for 5–60 minutes at room temperature. After five times, two-fold dilution series of the mixtures, they have incubated with an equal volume of 0.5% chicken erythrocyte (CRBC) suspension for another hour at room temperature for haemagglutination process (Nakayama et al., 1993).

3.4.4 Quantitative RT-PCR analysis

The vRNA was purified from MDCK cell culture by ultracentrifugation fractioning after being exposed to tea extracts. Then, cDNA had been synthesised from virus RNA, using appropriate reverse transcriptase and an influenza vRNA-specific, universal primer. Quantitative polymerase chain reactions had been performed under various multiplication conditions with a real-time PCR detection system. The resulted cDNA had been run in agarose gel electrophoresis after staining with ethidium bromide. Finally, the outcome was quantified as band intensities using a Gel Doc XR+ system (Kim et al., 2013).

3.4.5 Neuraminidase inhibition assays

Neuraminidase activity in virus towards catechin had been assessed in the process. Two-fold serial dilutions of catechin with PBS had been mixed with an equal volume of pre-decided influenza virus solutions. Another equal volume of the predetermined substrate solution was added to the mixture and been incubated at 37°C for two hours, in darkness. Finally, optical density had been measured by fluorescence of 4-methylumbelliferone contained in substrate solution, using a fluorescence spectrophotometer. Results had been quantified as ‘relative activities’ with the above measurements via the following formula:

$$\textit{Relative activities}\ (\%) = \frac{\textit{NA activities with catechins}}{\textit{NA activities without catechins}} \times 100.$$

3.4.6 Florescence microscopic techniques

Similar to neuraminidase inhibition assays accept a confocal microscope had been used to observe tagged cells instead of a fluorescence spectrophotometer. Same way MDCK cells had been exposed to virus at a pre-determined MOI (some had used 2.5 MOI while some research designs had preferred 100) and tagged by X-Neu5Ac and fast red violet LB according to test protocols conditions. Data obtained from images had been used to quantify and assess the impact of chemical constituents of tea (Müller and Downard, 2015).

In *metadata/social survey analysis* approach, two questionnaires had been given to a population before influenza season begins and after the influenza season. The first questioner had been consisting of information about their health state and second one

with information about their tea consumption behaviour and influenza infection details within the time period. Nor clinically monitored protocols such as gargling dosage prescriptions, pills nor biochemical/molecular biological tests had been done in this approach (Park et al., 2011).

Table 1 The summary of five clinical approach tests results.

Literature	*Nature of population*	*Statistical criteria*	*Individual count*		*Percentages*	
			Tea extract	*Placebo*	*Tea extract (%)*	*Placebo (%)*
Yamada et al. (2007)	Healthy adults	*Total sample numbers*	*195*	*200*	*49.4*	*50.6*
		Influenza illness	2	4	1.0	2.0
		Respiratory infections	94	103	48.2	51.5
Matsumoto et al. (2011)	Healthcare workers	*Total sample numbers*	*98*	*99*	*49.7*	*50.3*
		Clinically confirmed influenza	4	13	4.1	13.1
		Laboratory-confirmed influenza	1	5	1.0	5.1
Yamada et al. (2006)	Elderly nursing home residents	*Total sample numbers*	*76*	*48*	*61.3*	*43.9*
		Influenza illness	1	5	1.3	10.4
Ide et al. (2014)	High school students	*Total sample numbers*	*384*	*363*	*51.4*	*48.6*
		Clinically confirmed influenza	52	61	13.5	16.8
Toyoizumi et al. (2013)	High school students	*Total sample numbers*	*155*	*152*	*50.5*	*49.5*
		Influenza illness	11	12	7.1	7.9

3.5 Analysis of outcomes of different test approaches

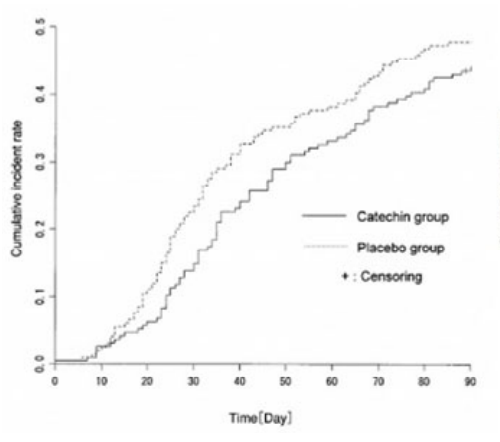
In clinical trials and diagnosis, approached research design results, the following summary of statistics had been observed (Table 1).

Based on the above statistics, the difference between individual values seemed insignificant to run statistical analysis in some tests to compare and contrast the impacts of green tea extracts or tea constituents on influenza infection. Even though the figures were smaller, still the green tea consumed group had shown slightly higher protection or resistivity against influenza. Based on the above statistics certain tests had shown 2–10 times of protection against influenza within in tea consuming groups compared to non-tea consuming groups. When the rate of cumulative cases being reported had been graphed over time as the Kaplan-Meier curve, tea constituents had shown promising results to prove that tea had been capable of giving a certain protection against influenza virus.

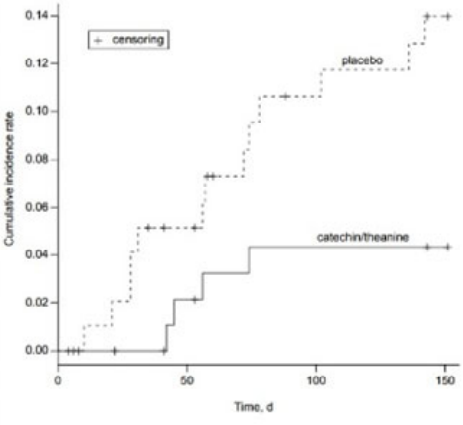
The rate of being infected by influenza had always been lower in groups who were consuming tea compared to non-tea consuming groups.

As for the in-vitro analysis results, it had shown promising results for tea constituents clearly reducing infection of influenza virus on used MDCK cells. The test had verified the results as well.

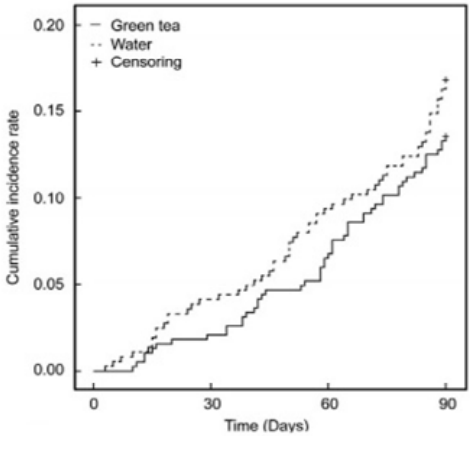
Figure 6 Kaplan-Meier curves for clinically-defined influenza in three different researches, (a) Yamada et al. (2007) (b) Matsumoto et al. (2011) (c) Ide et al. (2014)



(a)



(b)



(c)

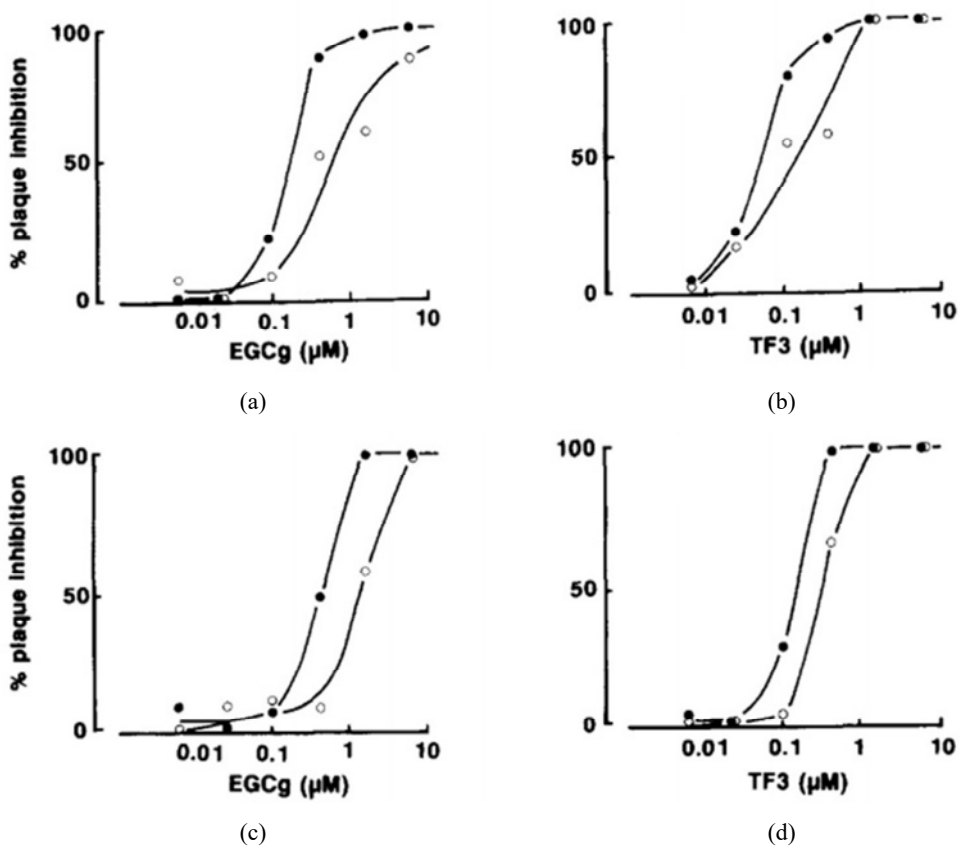
According to plaque inhibition assays, even low concentrations such as 1.5/μM of EGCG and TF3 compounds in tea had inhibited the infectivity of influenza viruses. The plaque-forming activity of the viruses had been inhibited almost by 100% after 60 min treatment.

In the other hand, the concentrations required to acquire maximum inhibition had not been significantly between two exposures time periods and simply five minutes exposure to substance would have effective reduce vial activity in cells.

Based on hemagglutination inhibition assays results, polyphenols in tea had been binding to HA antigens and been preventing virus adsorption into cells. TF3 had been more effective in this criteria.

When quantitative RT-PCR had been performed with MDCK cells infected with virus and exposed to tea constituents, using specific primers for vRNA (NP) and cellular RNA (actin), high concentrations of polyphenols mixture had shown most promising inhibitory effect (80%) not only in virus absorption but also on vRNA synthesis process.

Figure 7 Inhibitory effects of EGCG and TF3 on plaque formation, (a) influenza A virus in various concentrations of EGCG (b) influenza A virus in various concentrations of TF3 (c) influenza B virus in various concentrations of EGCG (d) influenza B virus in various concentrations of TF3



Note: (●) 60-minute exposure treatment and (o) 5 min exposure treatment

Source: Nakayama et al. (1993)

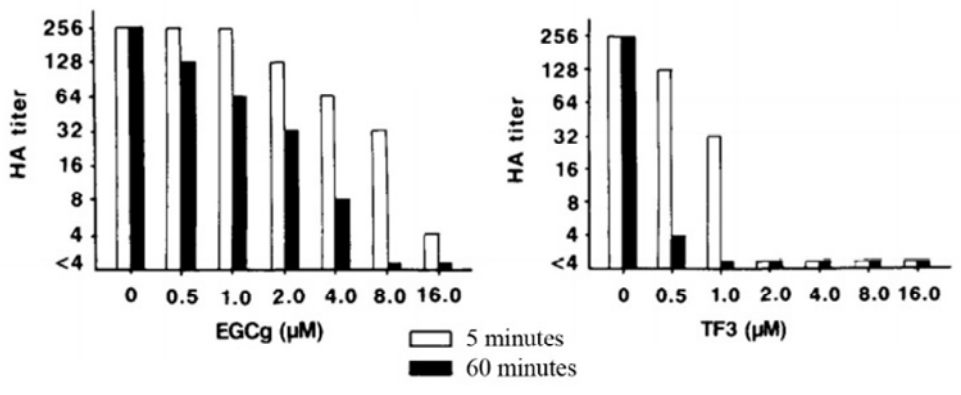
The band widths of gels in NP had been significantly smaller compared to controls which were not exposed to tea extraction active ingredients. These bands represent the quantity of RNA copies with in samples. If the viral activity is high and they replicate fast in cell, the viral gene copies are amplified in cells, this is detected by a high width of bands in controls with no treatments. However in treated groups, the gene amplification levels are significantly low. This implies Tea ingredients have positive effect on inhibition of viral replication in cells.

In neuraminidase assays, neuraminidase activity had been significantly intercepted by EGCG and ECG, but not by EGC at anticipated levels. But as a mixture, the combined effect had been promisingly effective on reducing neuraminidase activity.

50 μM of EGCG had been able to reduced virus yields by about four logarithmic units while at 100 μM concentration, complete inhibition had been achieved where no release of virus was detected, in a florescence microscopic assay. Based on this assay,

EGCG had been the most effective catechin tested, while ECG and C5G also had shown promising results on the reduction of virus yield.

Figure 8 Hemagglutination titers of influenza virus treated with various concentrations of tea constituents (a) EGCG or (b) TE3



(a) (b)

Source: Nakayama et al. (1993)

Finally, when virus yields had been quantified after being exposed to tea extracts, the virus yields observed in MDCK cells had been reduced by a scale of 2 log to 6 log units, depending on the concentration of the constituents used in the tests.

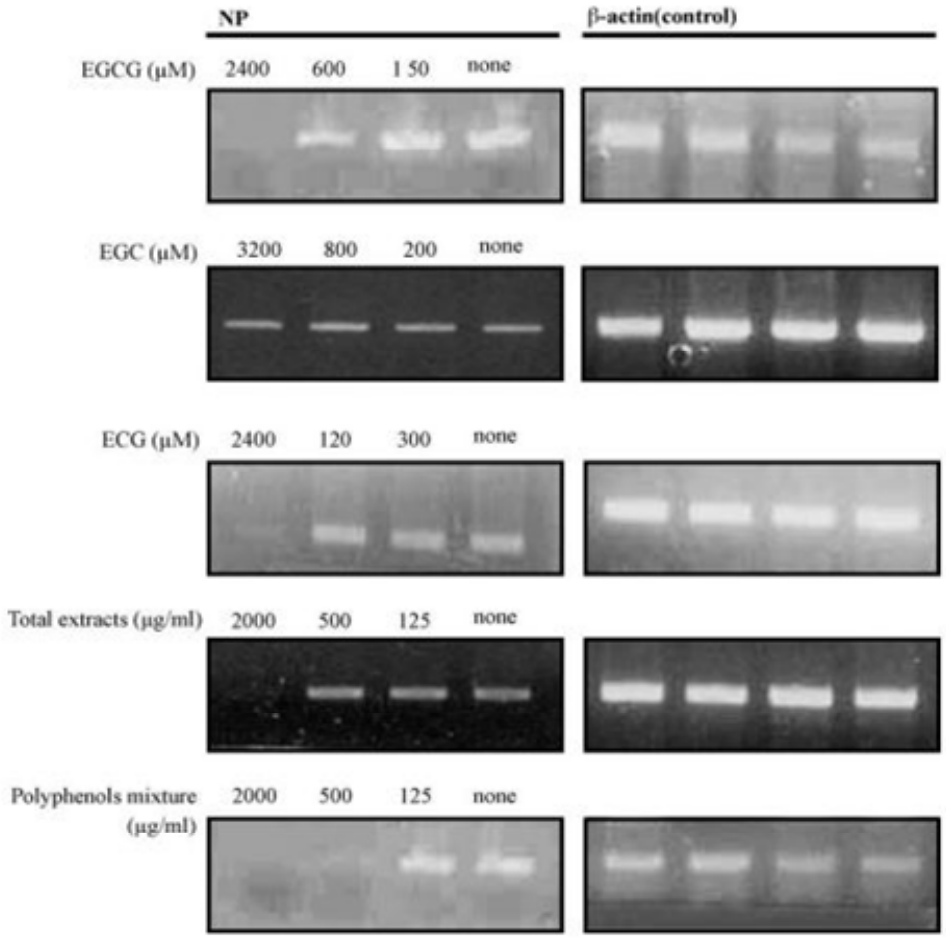
As per Figure 12, the reduction of viral yields in cells for total mixture and polyphenols mixtures had been approximately 50% in all exposure time lines. However when it comes to EGCG, it had been approximately 98% reduction and for ECG, it had been around 75%. These are significantly huge numbers to show the inhibitory effect of tea active ingredients against influenza.

But there are certain limitations between the theory or controlled in-vitro testing and reality in the natural human body.

Even though these approaches explain the final outcome and how it happens at cellular level in theory, there is a quite research gap on the pathway it happens in the real scenario. There are many questions to be answered between reality and fantasy such as:

- Once tea is ingested and digested, as what compounds it is absorbed into blood?
- What if they are purely not the same compounds such as EGCG, GCE, GEC, TF3, etc. that had been used for in-vitro testing, but different compounds that are synthesised into complex or simple molecules within the human body?
- As what molecules they reach to infected cells in human body in real conditions?
- Can polyphenols EGCG, TF3, etc. reach the infected target cells via blood or body fluid freely?

Figure 9 Effect of catechins on influenza vRNA synthesis in the infected cell as analysed by quantitative RT-PCR



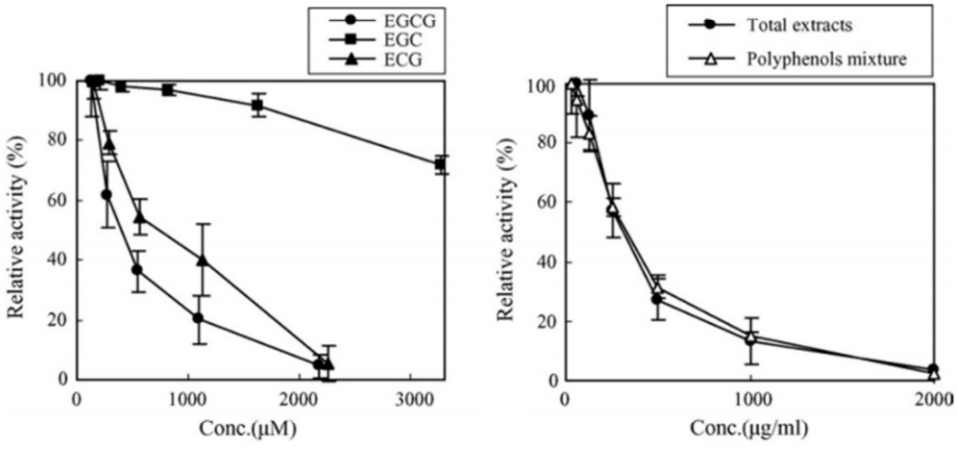
Source: Song et al. (2005)

Researching on living human specimens had been controlled by existing scientific etiquettes. Hence, research designs involving, infecting specimens with tagged virus genomes and administering tagged tea constituents for research purposes, etc. had been limited to conceptual approaches, making the above questions as gaps between clinical approach and molecular biological approach. Unfortunately, we could not find such a research design to answer those questions from biomedical aspect. Even though there would be such a rare design it would be extremely complicated to replicate it to check the precession of the procedure and results.

Even in the most accurate biochemical and molecular biological approach, MDCK cells are being used. As its name itself represent, the genome of cells represents *Canis lupus* specie. As recently discovered related to COVID-19, just having a gene cluster in size of 50 kilobases inherited by neanderthals, on chromosome 3 had increased the risk factor for Africans and Asians in COVID-19 infection (Zeberg and Paabo, 2020). *Canis*

is an entirely different specie related to human genome composition at the species level. The genomic effect of cell genome on cellular process related to influenza infection and immunity mechanism of MDCK cell could be different than human cells. Hence, the applicability of the conclusions drawn with MDCK cells on humans becomes questionable.

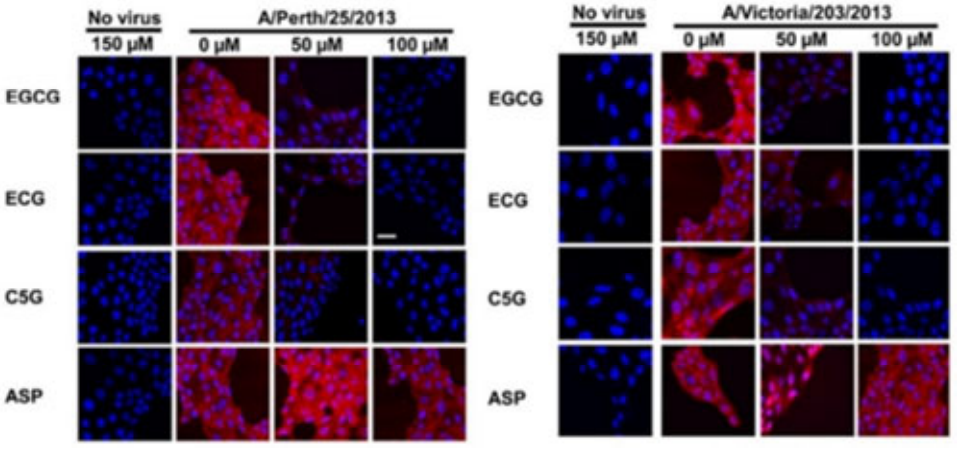
Figure 10 Inhibitory effects of viral neuraminidase activity by catechin constituents in tea, (a) individual effect of EGCG, EGC and ECG (b) combined effect of polyphenols mixture



(a) (b)

Source: Song et al. (2005)

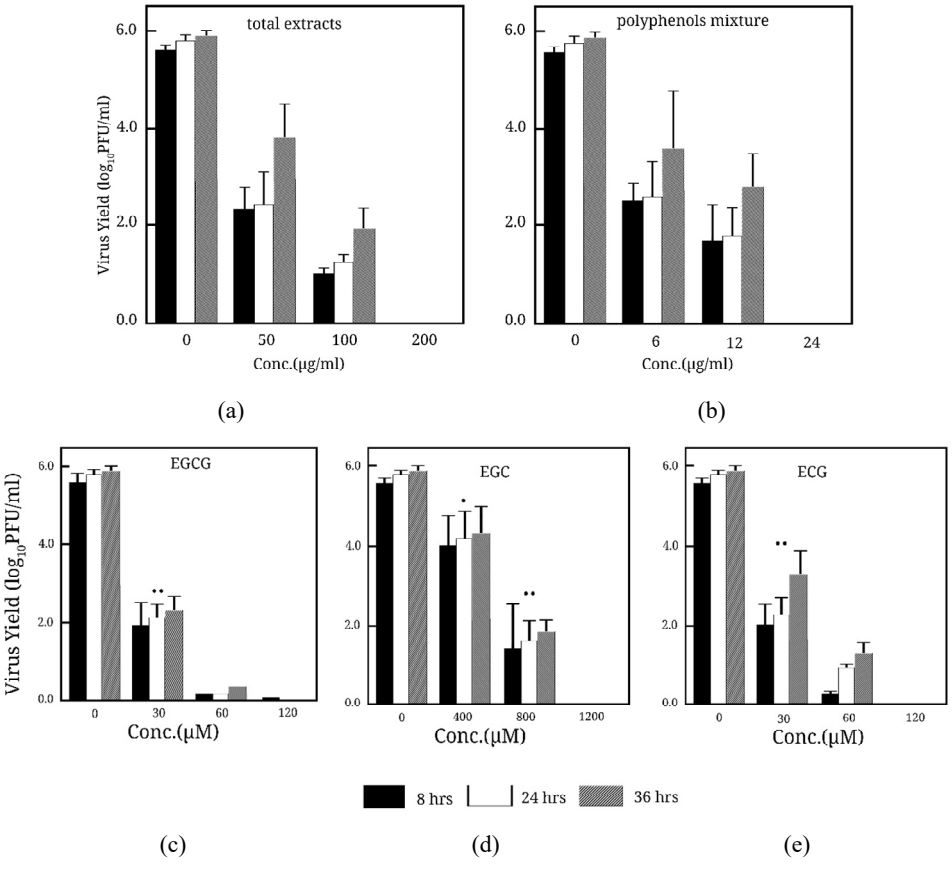
Figure 11 Fluorescent detection of neuraminidase activity of influenza virus strains by confocal microscopy after tagging with N-acetylneuraminic acid (X-Neu5Ac) and staining with fast red violet LB and DAPI, (a) A/Perth/25/2013 (b) A/Victoria/203/2013 virus strains (see online version for colours)



(a) (b)

Source: Müller and Downard (2015)

Figure 12 Inhibition effect of catechins on influenza virus yield in MDCK cells, (a) total extract effect (b) polyphenol mixture effect (c) EGCE effect (d) EGC effect (e) ECG effect



Source: Song et al. (2005)

Using organic fertilisers or animal faecal fertilisers such as panda faecal in China for tea cultivation had been an emerging trend and many literature had published that it could affect the flavour of the tea. This means that it must affect the chemical composition of tea somehow. But the anti-viral impact of this chemical alteration on the influenza virus had not been properly and scientifically explored yet in literature. Further, tea consists of hundreds of other chemical constituents. So far, literature had just tested the individual effect of those constituents or a maximum two or three at once. But when it comes to traditional medicine as well as modern pharmacological application, the combined effect of those minor unknown constituents could play a major positive or adverse impact on antiviral properties as well as cellular toxicology. These issues are not yet addressed properly in published literature so far.

4 Conclusions

When considering research designs and approach types there had been three main types of approaches. They are either clinical, biochemical, and molecular biological, or meta-analysis/social data analysis. From the above three, the clinical approach had simply shown that tea constituents affect reducing influenza infection with statistical evidences and the in-vitro biochemical and molecular biological approach had shown why and how it is happening with scientific laboratory-based evidence. Based on the accuracy, the biochemical and molecular biological approach is most accurate since it is tested under strictly controlled laboratory conditions. The clinical approach is moderately accurate since the clinical environment had been controlled to a certain extend. Even though the social survey approach is statistically okay, it could be less reliable since external factors such as administration dosage, exposure environments, risk factors, etc. are not controlled.

Based on the facts and figures obtained by literature, chemical compounds in tea have a significant impact on reducing infection of influenza.

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