Journal of Pharmacognosy and Phytochemistry 2015; 4(2): 282-291

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(2): 282-291
Received: 21-05-2015
Accepted: 25-06-2015

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Herbal green tea formulation using *Withania somnifera* stems, *Terminalia arjuna* bark, Cinnamon bark and *Tinospora cordifolia* stems and nutritional & phytochemical analysis

Parul Namdev, Rajinder K Gupta

Abstract
Tea in general and Green tea in particular, are gaining increasing consumer attention due to a growing awareness of health benefits derived from their use, but research in product development of flavored Green tea is limited. The objectives of the study were to conduct Nutritional analysis, phytochemical analysis, antioxidant and antibacterial activity of *Withania somnifera* stems, Cinnamon bark, *Tinospora cordifolia* stems, *Terminalia arjuna* bark, Green tea and formulation mixture of these herbs in order to assess their potential for new herbal tea development. These herbs are a rich source of natural antioxidants and polyphenols. Total phenolics and flavonoids contents were obtained for methanolic extracts for each herb sample. Antioxidant activities of the extracts were estimated using DPPH radical scavenging activity and FRAP assay. Formulation mixture is an excellent sources of calcium, magnesium and phosphorus. The methanolic extracts showed good antibacterial activity in *Bacillus subtilis, Bacillus cereus* and *E.coli.* GC/MS screening showed the presence of several useful compounds indicating that the samples hold the potential to be stated under "Nutraceuticals". HPLC method was developed for the estimation of quercetin, kaempferol, rutin & epicatechin in the formulation mixture extract. Formulation of green tea with these herbs was done, which imparts colour, aroma, flavor, astringency and overall acceptability to Green tea and impart many health benefits.

Keywords: *Withania somnifera* stems, Cinnamon bark, *Tinospora cordifolia* stems, *Terminalia arjuna* bark, Nutritional, Antioxidant, Phytochemical, Antibacterial, GC/MS

1. Introduction
The Tea is the most consumed beverage in the world, but its origination is attributed to china [1] and becomes as an important food product in the world. After china, the 2nd largest producer of tea is India [2].
These days consumers are cautious of their health so they are demanding for more natural and health benefitting food so tea seems to be a good vehicle in this respect because of its good taste and aroma. Hence tea belongs to a quickly growing market of wellness beverages [3].
Traditionally, tea is classified as green, Oolong, black and herbal tea [4] and can be differentiated on the basis of their processing stages at the time of manufacturing. *Camellia sinensis* is the plant from which green tea and all kinds of tea are made. Fermentation is not done during green tea Processing. Green tea imparts several health promoting components because of its high range of phenolic compounds. Green tea is covered to deliver nearly 4000 bioactive compounds under which one third is polyphenols [5].Tannins and flavonoids are the important polyphenols present in the Green tea. Catechins, one of the important flavonoid present in the green tea which is also known as vitamin P [6]. Green tea imparts several health benefits like weight loss, diabetes, heart diseases, alzheimer's and parkinson's, blood pressure, depression, anti-viral, skin care.
There are many flavored green teas. Popular flavored green teas are lemon green tea, ginger & mint green tea, lemon honey green tea, jasmine green tea, etc. It is thus imperative to research the potential of native plant materials in the expansion of new flavored green tea. On the basis of some unpublished reports, however, green tea imparts poor in sensory appeal due to the lack of distinct Flavor properties. Therefore, it may be needed to blend green tea with other herbs as a means of improving its sensory appeal and for good health and wellness. The herbs discussed in the research work are *Withania somnifera* stems, cinnamon bark, *Tinospora cordifolia* stems and *Terminalia arjuna* bark.
Withania somnifera (L) Dunal is fit to known as Indian medicinal plant which is usually used in the healing of many clinical conditions in India, also known as "Ashwagandha" contains a broad variety of significant substances in our bodies. It contains a large amount of steroidal

alkaloids like anferine, somnine and withanolides, iron, fatty acids, antioxidants like glyco-withanolides and potassium nitrate. The constituents present in the leaves of this herb widely used in the ayurvedic medicines. While the roots also contain the good amount of active compounds which are useful in several health diseases. Ashwagandha has been found to give strong antioxidant protection [7], boosts up the immune system cells, such as lymphocytes and phagocytes [8, 9] & counteract the effects of stress and normally imparts wellness [10].

Cinnamon bark of a variety of cinnamon species is one of the most important and accepted spices used worldwide not only for cooking, but as well in traditional and modern medicines [11, 12]. Cinnamon is mostly used in the aroma and essence industries due to its fragrance, which can be integrated into varieties of food stuffs, perfumes, and medicinal products [13]. Cinnamon involves a variety of beneficial many compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and essential oil [14]. Cinnamon has several health benefits such as anti-inflammatory [15, 16], antitermitic, nematicidal [17, 18], mosquito larvicidal [19], insecticidal [20], antimycotic and anticancer agent [21, 22].

Tinospora cordifolia (Giloy) is one of the vital medicinal plants and an essential drug of the Ayurveda. This plant also known as Amrita or Guduchi. This herb involves the different types of active constituents such as alkaloids, glycosides, steroids, phenolics, aliphatic compounds and polysaccharides [23]. It also contains Flavonoids, saponins and several amount of phytosterols. These all compounds impart the antioxidant activity [24]. The leaves of this herb consist good amount of protein and a fair amount of phosphorus and calcium [25, 26]. It is used for antioxidant, anticancer, immunostimulant, and antidiabetic properties.

Terminalia arjuna is a tree in which its bark is having a good medicinal properties. It acts as a cardiotonic. It is helpful to lower down the blood pressure and pulse rate, and may boost up the aerobic exercise capacity. Major chemical present in the bark is saponins, flavonoids, tannins and phytosterols. It has antibacterial antimutagenic, antioxidant, hypolipidemic, and hypocholesterolaemic and anti-inflammatory effects [27].

The aim of the present study is to evaluate the nutritional, phytochemical constituents, antioxidant and antibacterial activity of *Withania somnifera* stem, Cinnamon bark, *Tinospora cordifolia* stems, *Terminalia arjuna* bark and Formulation mixture and to blend these herbs with the Green tea.

2. Materials and methods

2.1. Sample collection

Withania somnifera stem, Cinnamon bark, *Tinospora cordifolia* stems and *Terminalia arjuna* bark, Green tea were collected from a local market of Chandni Chowk, New Delhi. All herbs were carefully inspected and all foreign materials removed and thoroughly washed under tap water. The clean sample was dried in an oven at 60 °C and coarsely powdered using a mixer grinder, sieved and then stored in air- tight, light resistant container for further use. The samples were labelled as the *Withania somnifera* stem (WS), *Terminalia arjuna* bark (TA), Cinnamon bark (C), *Tinospora cordifolia* stems (TC), Green tea (GT) and Formulation mixture (F MIX) respectively.

2.2. Sample extraction

About 25g each of the powdered herb samples (WS, TA, C, TC, GT) and 50g of powdered F MIX of these herbs was successively extracted with 100 ml and 200 ml of methanol respectively. The mixture was incubated at 60 °C at 150 rpm for 24 hrs in an incubator shaker. The mixture was filtered through Whatman paper to obtain the filtrate. The residue left after the methanol extraction was again mixed with another 100 ml and 200 ml methanol respectively, and then again incubated at 60 °C at 150 rpm for 24 hrs in an incubator shaker for 24 hrs and then both the combined filtrates obtained were then transferred to respective labelled beakers and covered with foil, with fine pores for the solvent to evaporate at 60 °C in a hot air oven to afford methanol extracts.

2.3. Nutritional Analysis

2.3.1. Determination of Moisture content

The moisture content was measured described by AACC [28] method. Two gram sample was placed in a preheated and weighed glass petriplate and then dried in a hot air oven at 130 °C for 2 hrs or till constt. Weight after drying glass petriplate was transferred to the dessicator to cool and then petriplate was reweighed. The loss in weight was calculated as percentage of moisture content.

$$\text{Moisture content (\%)} = \frac{W1 - W2}{\text{Weight of Sample}} \times 100$$

W1 = Weight (g) of Sample before drying.
W2 = Weight (g) of Sample after drying.

2.3.2. Determination of Ash content

The ash content was measured, described by AACC [28] method. Two gram sample was placed in a preweighed crucible and then uncovered crucible was allowed to incinerate in a muffle furnace at 820 °C for 4 hours and then crucible was cooled in a desiccator and then weighed.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of Sample}} \times 100$$

2.3.3. Determination of mineral composition

Inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature in a range from 6000 to 10000 K. It is also a solution technique & standard silicate dissolution methods are employed [29]. The intensity of this emission is indicative of the concentration of the element within the sample.

2.4. Phytochemical analysis

2.4.1. Determination of Total Phenolics

Total phenolics were determined using the Folin–Ciocalteu assay [30]. The absorbance was measured at 765 nm and the results were expressed in gallic acid equivalents.

2.4.2. Determination of Total Flavonoids

Total flavonoids were estimated using Aluminiun Chloride colorimetric method [31]. The Absorbance was measured at 510 nm. Results were expressed in Catechin equivalents.

2.4.3. Determination of Crude alkaloids

Crude alkaloid was determined gravimetrically for phytochemical analysis [32] with some modification. The alkaloid content was calculated as a percentage.

$$\% \text{ of Alkaloid} = \frac{\text{Final Weight} - \text{Initial weight}}{\text{Sample Weight}} \times 100$$

2.4.4. Determination of Tannins

Tannins were estimated according to the method described by Van-Buren and Robinson [33]. Absorbance was measured at 605nm and the result were expressed in Tannic Acid equivalents.

2.5. Qualitative phytochemical analysis

The methanolic extracts of WS, C, TC, TA, GT & F MIX were subjected to different chemical tests for the detection of phytoconstituents such as terpenoids, phytosterol, coumarin, anthraquinone, phlobatannins.

2.5.1. Test for identification of Terpenoids

5 mg of the methanol extract was mixed with 2 ml of chloroform and 2 ml concentrated sulphuric acid. The layer interface was observed for reddish brown coloration which indicates the presence of terpenoids [34].

2.5.2. Test for identification of Coumarins

10 mg of the extract is dissolved in methanol and alcoholic KOH was added. The appearance of yellow colour which decolorizes while adding conc. HCl shows the presence of coumarin [35].

2.5.3. Test for identification of phytosterols

Small quantities of various extracts were dissolved in 5 ml of chloroform separately then chloroform solution was subjected to following test: Salkowski test: - To 1 ml of above prepared chloroform solution, a few drops of conc. H_2SO_4 was added. Brown colour produced showed the presence of phytosterol [34].

2.5.4. Test for identification of phlobatannins

80 mg of plant extract was boiled in 1% HCl, the deposition of a red precipitate indicated the presence of phlobatannins [36].

2.5.5. Test for identification of anthraquinone

Two hundred mg of fraction was boiled with 6 ml of 1% HCl and then filtered. Then the filtrate was shaken with 5 ml of benzene. Layer of benzene was removed and 10 % ammonium hydroxide was added. Pink, violet and red in alkaline phase indicates the presence of anthraquinone [37].

2.6. Antioxidant activity

2.6.1. DPPH radical scavenging activity

DPPH radical scavenging activity was analyzed by the method of Blois [38] with slight modification. Two mg (200 PPM) of sample extract was dissolved in 10 mL methanol and then from this 10 mL, 1mL of sample was taken and dissolved with 1mL of a 0.3 Mm methanol solution of DPPH (2, 2-diphenyl-1 –picrylhydrazyl) plus 1 mL methanol in a test tube. After this test tube was kept in the dark for 10 minutes. Blank was prepared without the extract. Methanol was used as a reference. Then absorbance was taken at 517nm.

A radical scavenging activity was expressed by % of scavenging activity and was calculated by the following formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{OD Blank} - \text{OD Sample}}{\text{OD Blank}} \times 100$$

2.6.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined by Benzie and Strain [39] method. The FRAP reagent was prepared fresh each day by mixing 2.5 mL of TPTZ (10 mM in 40 mM hydrochloric acid), 2.5 mL of ferric chloride (20 mM) and 25 mL of sodium acetate buffer (300 mM, pH 3.6). A 100 µl of extract was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37 °C for 4 min. The absorbance was measured at 593 nm and the result was expressed as BHT equivalent.

2.7. Determination of antibacterial activity

The antibacterial activity of the Methanolic extract of F mix was evaluated by agar well diffusion method against three Gram positive bacteria and two Gram negative bacterial test pathogens [40]. Extracts were reconstituted to a final concentration of 100mg/ml and 500 mg/ml. DMSO and Methanol were used as control in one of the wells. Nutrient agar was inoculated for each bacterial strain by spreading each strain 100 µl in the respective plate. Wells of 6 mm diameter were punched on the agar plates and 100 µl of extracts were loaded into the wells. The plates were incubated at 37 °C for 24 hrs. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and reported on the scale of millimeters (mm).

2.8. GC-MS Analysis

The Gas chromatography mass spectrometry (GC-MS) analysis of extracts of WS, C, TC, TA, GT, F MIX were performed by using 1µl each, of methanolic extract of the sample. The carrier gas was Helium. An Agilent 6890 GC with 5975B MSD (mass spectrometry detector) was used in the scan mode (m/z 35-1050) designed for the sample. Automatic RTL screener software in arrangement with the Agilent NIST'05 library used for Screening of volatiles and semi volatiles [41]. The temperature for the analysis was maintained at 300 °C, solvent delay was 3 min, ion source and quadruple temperature were 230°C and 150 °C, respectively. The detected compounds have been identified by the NIST'05 mass spectrum library. The identity of the secondary metabolites in the methanol extracts of these herbs & their mixture was carried out by Mass Spectroscopy based on the comparison of their retention time.

2.9. HPLC analysis

High performance liquid chromatography method is an accurate, precise, simple and specific method which was developed for the simultaneous estimation of quercetin and kaempferol in an F MIX of herbal extracts (WS, C, TC, TA & GT).

2.9.1. HPLC instrumentation

Agilent infinity 1200 series system comprising a pump, an automatic sampler and a diode array (DAD) detector was used with data acquisition by EZ chrome elite software.

2.9.2 Preparation of reference solution for quercetin, kaempferol, epicatechin and rutin.

A stock solution of reference standard of quercetin, kaempferol, epicatechin and rutin were arranged by accurately weighing about 1mg quercetin and kaempferol standard in 5 ml of methanol in a volumetric flask and sonication for 5-10 minutes.

2.9.3. Preparation of test solution

400 mg sample (F MIX extract) were accurately weighed and dissolved in 5 ml of methanol in a volumetric flask along with sonication for 5-10 minutes followed by the filtration of contents of the flask.

2.9.4. Chromatographic system for quercetin and kaempferol

The following conditions for the quantification of quercetin and kaempferol was developed by Indian Pharmacopeia [42]

- Stationary phase- a stainless steel column 25cm x 4.6mm packed with octadecylsilane bonded to porous silica (5µm).
- Mobile phase- a mixture of 40 volumes of acetonitrile and 60 volumes of buffer solution prepared by dissolving 3.01g of potassium di-hydrogen orthophosphate in 1000 ml of milli Q water and pH 2.0 was adjusted with acetic acid
- Flow rate-1 ml/minute.
- Spectrophotometer sets at 370 nm.
- Injection volume-10µl.
- Run time-15 minutes.
- Column temperature-25 °C

2.9.5. Chromatographic system for epicatechin and rutin

The following conditions for the quantification of epicatechin and rutin was developed by Nour *et al.* [43]

- Stationary phase- a stainless steel column 25cm x 4.6mm packed with octadecylsilane bonded to porous silica (5µm).
- Mobile phase-solvent A 1% acetic acid and solvent B methanol
- Flow rate-1 ml/minute.
- Spectrophotometer sets at 278 nm.
- Injection volume-10µl.
- Run time-15 minutes.
- Column temperature-25 °C

Reference and test solution were injected.

Formula for the content of quercetin, kaempferol, epicatechin and rutin:

$$(\%) = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard (mg)}}{\text{Standard dil.}} \times \frac{\text{Sample dil.}}{\text{Sample (mg)}} \times \frac{\text{Standard potency}}{100}$$

2.10. Preparation of Formulations

The five dried and milled herbs (WS, C, TC, TA & GT) were mixed in varying proportions to obtain three different formulations Table 1, 1.75 gram samples of each formulation were bagged in rectangular infusion tea bags. Commercial Green tea (Tetley Company) was used as a control. They were labelled accordingly for sensory analyses.

Table 1: Proportion of herbs in blended product

Herbs	Formulation 1 (%)	Formulation 2 (%)	Formulation 3 (%)	Control
GT	30	40	50	100
WS	25	15	15	-
TC	20	20	15	-
C	5	10	5	-
TA	20	15	15	-

2.11. Sensory evaluation

Sensory evaluation is a scientific regulation used to determine those characteristics of food and materials as they are perceived by the senses of touch, smell, sight, and hearing. Sensory profiling is descriptive methods that qualifies and quantifies organoleptic properties of products. Quality attributes (color, texture, taste) of prepared fortified Green tea were evaluated by twenty members.

3. Results and Discussion

3.1. Nutritional analysis

Moisture content and ash analysis during nutritional analysis are very important because it directly affects the nutritional content of the food, its stability and storage, etc. The moisture and ash contents were calculated for F MIX which are reported in Table 2. The mineral components were analyzed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). The quantity of minerals present in the F MIX is as tabulated in the Table 3. The good amount of calcium was present in F MIX which can play a vital function in rigidity to the bones as well in the neuromuscular functions, blood clotting, treatment of osteoporosis [44] whereas magnesium has multiple health benefits including transmission of nerve impulses, detoxification, body temperature regulation, energy production, heart diseases [45] and the development of fit bones and teeth. Another one is phosphorous, which were present in high amount. The potency of phosphorous comprises strong bone formation, enhanced digestion, regulated excretion, improved energy extraction, hormonal balance, protein formation, cellular repair, and also crucial for performing vital actions for different body parts like the brain, kidney, heart and blood [46].

Table 2: Ash and Moisture content estimation for F MIX

Constituents	(F MIX)
Moisture	5 %
Ash	8.1 %

Table 3: Mineral content estimation for F MIX

S no.	Analyte	Concentration (ppm)
1	Ca	9985.50
2	Fe	173.75
3	Mn	167.50
4	Mg	1040
5	Ni	2.13
6	P	13925.0
7	Sr	45.0
8	Zn	15

3.2. Phytochemical analysis

Phenolics and Flavonoids are a wide class of chemical compounds found in plants. They impart quality and nutritional value and plays a vital role in human fitness such as anti-inflammatory [47], antidibatic [48], antiviral [49], antioxidant [50], Therefore, total phenolic and flavonoid content of different methanolic extracts of herbs were estimated Table 4. Crude alkaloids and tannin are the compounds present in plants. Tannins are polyphenols that are responsible for the astringent flavor of food and shows anti-carcinogenic [51] and antimicrobial properties where as small doses of alkaloids have a therapeutic effect as muscle relaxants, pain killers and antimicrobials in human beings [52]. Therefore, crude alkaloid and tannin content of different samples of herbs were estimated Table 4.

Table 4: Polyphenolic compounds of different methanolic extracts

Herbal extracts	GAE equivalents (µg GAE/mg sample)	Catechin equivalents (µg CE/ mg)	Alkaloids (%)	Tannic acid equivalents (µg TAE/mg)
WS	7.8	24	0.804	0.1235
TA	41.25	84	2.016	0.0785
C	3.43	120	1.172	0.2985
TC	4.68	42	0.368	0.2985
GT	30.31	58	0.672	0.2335
F MIX	18.43	50	1.56	0.271

3.3. Qualitative phytochemical analysis

Methanolic extracts of WS, C, TC, TA, GT & F MIX were also subjected for qualitative tests such as phytosterol, terpenoids, anthraquinone, coumarin and phlobatannins in which all the herbs show the presence of phytosterol and terpenoids in Table 5. Phytosterols are very beneficial in lowering cholesterol levels and reduces coronary heart diseases [53] while terpenoids have a unique antioxidant activity [54]. Coumarin were found to be present only in WS and F MIX which shows the anti-fungal and anti- tumor activity.

Table 5: Qualitative analysis of herbal extracts to screen the presence of phytochemicals**.**

Sample	Phytosterol	Terpenoids	Phlobatannins	Anthraquinone	Coumarin
WS	+	+	-	-	+
TA	+	+	-	-	-
C	+	+	-	-	-
TC	+	+	-	-	-
GT	+	+	-	-	-
F MIX	+	+	-	-	+

3.4. Antioxidant activity Analysis

3.4.1. DPPH radical scavenging activity

This method is most commonly used for screening of antioxidant activity of many herbal plants. DPPH is a stable free radical with violet colour. If free radicals have been scavenged, DPPH will change its colour from violet to pale yellow or colourless. This property allows visual monitoring at 517 nm. A scavenging activity in % inhibition of methanolic herbal extracts is given in Table 6. Antioxidants are working as a protection of cells against the destructive effects of reactive oxygen species (super oxide or hydroxyl radicals). The reaction between antioxidants and reactive oxygen species results in oxidative stress, causes cellular damage [55]. Oxidative stresses have been related to cancer, aging, atherosclerosis, inflammation, ischemic injury and Neuro degenerative diseases [56].

Table 6: DPPH assay of different herbal extracts

Analyte	% Inhibition
WS	51.95
TA	87.93
C	38.91
TC	45.46
GT	94.10
F MIX	93.29

3.4.2. Ferric reducing antioxidant power (FRAP) assay

This method is based on the principle of reduction of ferric tripyridyl-s-triazine complex to ferrous colored form in the presence of antioxidants. The Antioxidants present in the samples reduces ferric tripyridyl-s-triazine complex to form a blue colored complex which results in an increase in the absorbance at 593 nm. The antioxidant acitivity of different methanolic herbal extracts is given in Table 7.

Table 7: FRAP assay of different herbal extracts

Analyte	BHTE equivalents(µg BHTE/mg extract)
WS	861
TA	2930
C	1876
TC	961
GT	2223
F MIX	2930

3.5. Antibacterial activity

Four gram positive *(Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis*) and two gram negative (*E. coli, Salmonella enterica*) bacteria were used to determine the antibacterial activity of methanolic extract of F MIX. Agar well diffusion method was used to

determine the activity against the bacteria by measuring zone of inhibition. Inhibiting concentrations used for the extract was 100mg/ml and 500 mg/ml. Antibacterial activity was analyzed against the DMSO and methanol solvents. The zone of clearance or zone of inhibition of methanolic extract of the F MIX against various bacterial growths is listed in the Table 8.

Table 8: Antibacterial activity of F MIX against various bacteria using the agar well diffusion method

Test pathogens	Diameter of zone of inhibition (mm) Methanolic extracts			
Solvents	**DMSO**		**Methanol**	
Gram positive	500mg/ml	100mg/ml	500mg/ml	100mg/ml
Bacillus subtilis	25	14	28	15
Bacillus cereus	22	15	23	19
Staphylococcus aureus	15	11	21	15
Staphylococcus epidermidis	15	1	20	11
Gram negative				
E.coli	23	14	22	13
Salmonella enteric	22	15	23	19

3.6. Secondary metabolites estimation

The secondary metabolites in the samples were determined by GC-MS. Analysis of methanolic extract of WS, C, TC, TA, GT and F MIX resulted in identification of phytochemicals and common fatty acid. The details of some identified compounds are grouped according to their chemical nature. The main compounds were tetradecanoic acid, which is also known as Myristic acid (common fatty acid) that is found in both animal fats and plant oils. Myristic acid is used in the food industry as a flavoring agent and as a fragrance ingredient; one of its primary properties is as a lubricant, due to its high rate of absorption by the skin. Another compound is hexadecanoic acid, also called as palmitic acid, which is the most common saturated fatty acids found in plants. This compound possesses mild antioxidant [57] and anti-atherosclerotic properties. The presence of phytol, stigmasterol and campesterol which are commonly used in medical, cosmetic, and functional food applications, may impart antimicrobial and antioxidant activities. They are as well recognized for their saturated fat reducing and cholesterol lowering action and therefore, may kill the hazard of heart diseases. Phytol can be used as a precursor for manufacturing synthetic forms of vitamin E and vitamin K. Phytol is also used in various industries like fragrance, cosmetics, shampoos, toilet soaps, household cleaners, and detergents [58]. Vitamin E and tocopherol were found. Vitamin E is very important for strong immunity, healthy skin, eyes and shows an antioxidant activity [59]. Another compound is α-amyrin is a natural chemical compound of the triterpene class, widely distributed in nature and have been isolated from a variety of plant sources which shows several health benefits as anti-inflammatory, antioxidant, gastroprotective and hepatoprotective effects at non-toxic doses [60].

Table 9: GCMS profiling of methanolic extract of WS

Compound name	CAS#	RT	% Area
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	028564-83-2	9.917	1.82
2-Furancarboxaldehyde, 5-(hydroxym ethyl)	000067-47-0	11.498	4.43
8-Azabicyclo[3.2.1]octan-3-ol, 8-m ethyl-, endo	000120-29-6	11.891	2.75
n-Hexadecanoic acid	000057-10-3	20.280	5.95
Heptadecanoic acid	000506-12-7	21.177	0.58
9,12-Octadecadienoic acid, methyl ester	002462-85-3	21.480	1.05
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	21.929	9.74
Cyclohexanol, 5-methyl-2-(1-methyl ethenyl)	007786-67-6	23.162	0.57
Eicosanoic acid	000506-30-9	23.846	1.36
Campesterol	000474-62-4	33.447	1.13
gamma.- Sitosterol	000083-47-6	35.376	0.78

Table10: GCMS profiling of methanolic extract of TA

Compound name	CAS#	RT	% Area
4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	028564-83-2	10.018	0.43
gamma.-Sitosterol	000083-47-6	35.409	4.84
9-Octadecenoic acid (Z)-, methyl ester	000112-62-9	21.536	0.82
Hexadecanoic acid, methyl ester	000112-39-0	19.854	0.42
n-Hexadecanoic acid	000057-10-3	20.291	2.77
9,17-Octadecadienal, (Z)-	056554-35-9	21.973	4.36

Table 11: GCMS profiling of methanolic extract of C

Compound name	CAS#	RT	% Area
Glycerin	000056-81-5	7.865	1.45
Benzoic acid, methyl ester	000093-58-3	8.841	0.31
Benzenepropanal	000104-53-0	10.108	0.31
Benzenecarboxylic acid	000065-85-0	10.556	0.25
Cinnamaldehyde, (E)	014371-10-9	12.059	16.22
2-Propen-1-ol, 3-phenyl-	000104-54-1	12.542	1.58
alpha.-Cubebene	017699-14-8	13.484	1.94
2H-1-Benzopyran-2-one	000091-64-5	14.549	9.15
2-Dodecen-1-ol	022104-81-0	16.456	1.05
Tetradecanoic acid	000544-63-8	18.183	0.50
n-Hexadecanoic acid	000057-10-3	20.280	3.56
Vitamin E	010191-41-0	31.608	0.72
Stigmasterol, 22,23-dihydro	1000214-20-7	35.376	1.58

Table 12: GCMS profiling of methanolic extract of TC

Compound name	CAS#	RT	% Area
Phenol, 2-methoxy-	000090-05-1	8.807	0.38
2-Propenal, 3-phenyl	000104-55-2	12.003	0.62
2-Methoxy-4-vinylphenol	007786-61-0	12.631	0.66
Vanillin	000121-33-5	13.966	1.08
Phenol, 2-methoxy-4-(1-propenyl)-, (Z)	005912-86-7	14.549	0.85
Tetradecanoic acid	000544-63-8	18.172	1.14
n-Hexadecanoic acid	000057-10-3	20.314	9.73
Phytol	000150-86-7	21.659	4.59
Oleic Acid	000112-80-1	22.960	0.68
Eicosanoic acid	000506-30-9	23.835	1.78
Cyclopentadecane	000295-48-7	24.845	1.54
Vitamin E	000059-02-9	31.596	1.44
Campesterol	000474-62-4	33.436	1.88
Stigmasterol	000083-48-7	34.075	2.03
alpha.-Amyrin	000638-95-9	36.228	2.57

Table 13: GCMS profiling of methanolic extract of GT

Compound name	CAS#	RT	% Area
1,2-Benzenediol, 3-methoxy-	000934-00-9	11.981	0.56
1,2,3-Benzenetriol	000087-66-1	13.999	12.72
Tetradecanoic acid	000544-63-8	18.183	0.47
n-Hexadecanoic acid	000057-10-3	20.280	2.71
Squalene	007683-64-9	27.559	1.00
Vitamin E	010191-41-0	31.607	2.54

Table 14: GCMS profiling of methanolic extract of F MIX

Compound name	CAS#	RT	% Area
Glycerin	000056-81-5	7.854	0.78
4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	001073-96-7	10.702	0.23
1,2-Benzenediol, 3-methoxy	000934-00-9	11.936	0.59
Copaene	003856-25-5	13.472	0.24
1,2,3-Benzenetriol	000087-66-1	14.123	9.33
Dodecanoic acid	000143-07-7	15.929	0.77
Tetradecanoic acid	000544-63-8	18.183	0.35
Pentadecanoic acid, 14-methyl-, me thyl ester	005129-60-2	19.843	2.30
n-Hexadecanoic acid	000057-10-3	20.314	3.38
Phytol	000150-86-7	21.660	2.29
Heptadecanoic acid	000506-12-7	21.189	0.52
Eicosanoic acid	000506-30-9	23.824	0.65
Squalene	007683-64-9	27.559	1.16
Vitamin E	000059-02-9	31.596	1.60
Campesterol	000474-62-4	33.413	0.53

3.7. HPLC analysis

HPLC is one of the best separation techniques of chromatography showing the concentration of four (quercetin, kaemperol, epicatechin & rutin) different phytochemicals in the F MIX. It is also known as high pressure liquid chromatography, is basically a form of column chromatography in which the (stationary phase) length column 2-30 cm in which silica particle size is 2-10 µ and the diameter of the column is 3.5-4.5 mm.

The study of quercetin and kaempferol was conducted on HPLC infinity 1200 series system quipped with injector and DAD (Diode array detector) detector for the quantitative estimation of quercetin and kaempferol from methanolic F MIX extract with a stainless steel column 25cm x 4.6mm packed with octadecylsilane bonded to porous silica (5µm) as a stationary phase and mobile phase for quercetin and kaempferol is a mixture of 40 volumes of acetonitrile and 60 volumes of buffer solution (potassium di-hydrogen orthophosphate). HPLC spectra of standard quercetin and kaempferol shows peak at retention time of 5.387 & 8.127 minutes respectively which is represented in Fig 1. The concentration of quercetin, kaempferol, epicatechin & rutin is given in Table 15.

Quercetin is a valuable in the nutritional management of diabetes. It shows the excellent properties of antioxidant and antidiabetic which protects against coronary heart disease (CHD) [61], while kaempferol and some glycosides of kaempferol have a pharmacological activity like antioxidant and antidiabetic, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective [62]. The analysis of epicatechin and rutin was conducted by using mobile phase comprised of solvent A and solvent B in which solvent A was 1.0% acetic acid and solvent B was methanol. The standard solution and the test solution were injected in HPLC. (-)-Epicatechin and rutin showed peak at retention time 4.207 & 6.267 minutes respectively which is represented in Fig 2.

Epicatechin and rutin were valuable for its antidiabetic properties and also act as an antioxidant. Rutin is a bioflavonoid. It is found in many plants, fruits and vegetables. Rutin has strong antioxidant properties [63] when used with vitamin E, may help fight high cholesterol.

Table 15: HPLC analysis of phytochemical compounds in F MIX methanolic extract.

Phytochemical	Retention time	Area	Concentration (%)
Quercetin	5.387	377531	0.6023
kaempferol	8.127	134609	0.4913
epicatechin	4.207	3552868	0.1983
Rutin	6.267	1111460	0.2462

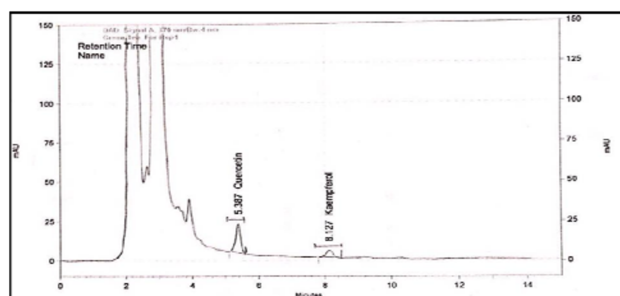

Fig 1: Estimation of quercetin and kaempferol in F MIX extract

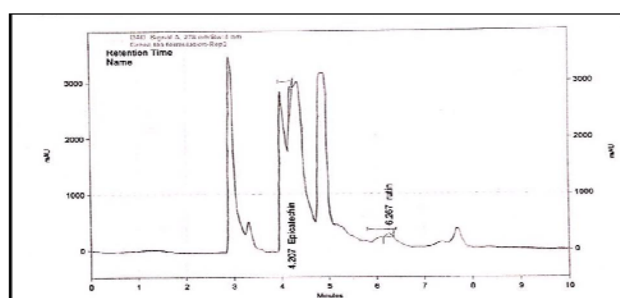

Fig 2: Estimation of epicatechin and rutin in F MIX extract

3.8. Sensory properties of formulated green tea
The three formulations products were presented to each member in 50 ml of hot water. The result showed Fig 3 that 3rd formulation received the highest sensory. Sensory evaluation of tea was done for overall acceptance (colour, aroma, Flavor, astringency) by 10 members on the basis of 9-point Hedonic scale.

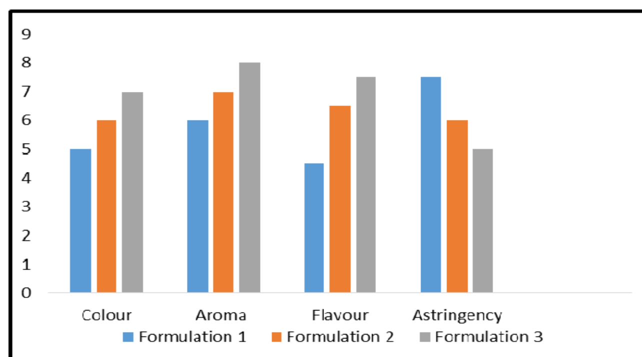

Fig 3: Sensorial attributes of Formulated Green Tea

4. Conclusion
The results of the nutritional, phytochemical, antioxidant and antibacterial activity showed that *Withania somnifera* stem, Cinnamon bark, *Tinospora cordifolia* stems, *Terminalia arjuna* bark, Green tea and the formulation mixture of these herbs showed that they can be proven to be an excellent source of nutraceuticals and Flavoring agents. Multiple health benefits featured in the blended formulation make it a perfect physical and psychological health Rejuvenator. Although several health benefits are also credited to green tea, but according to some unpublished reports it has been observed that the sensory appeal of green tea is not much attractive due to the lack of distinct flavor properties. It may therefore can be a good idea to combine green tea with other herbs (*Withania somnifera* stem, Cinnamon bark, *Tinospora cordifolia* stems and *Terminalia arjuna* bark) for developing flavored green tea, which not only adds to its appeal, but also palatability & thereby making it a wonder product in the context of human health. As sensory appeal matters the most to consumers more than health or nutritional benefits, so the above infusion will provide them with new alternatives to traditional flavored teas which can impart health benefits too.

5. Acknowledgement
We are very grateful to the University Grants commission for the financial support under the Special Assistance Program (SAP).

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