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PREPARATION OF ANTIOXIDANT POLYHERBAL TEA

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ABSTRACT

The most popular and effective natural remedy today is an infusion or decoction made from the beneficial parts of plants and herbs, such as their blossoms, leaves, and roots. Herbal teas are frequently used in the treatment of painful conditions like cancer, digestive problems, and decreased glucose tolerance.Herbal teas were made using infusion or decoction techniques in accordance with the instructions on their labels (Table 1). Infusion: Teas were prepared using water; 100 ml of deionized hot water was added to a given quantity of tea, and the mixture was allowed to steep for 3 minutes without further heating. Decoction: A precise amount of tea was cooked in 100 ml of cold, deionized water for 15 minutes, followed by a 10-minute pause. All the samples were run through Whatman No. 4 filter paper before being 55 °C vacuum- concentrated to a final volume of 50 ml. The polyherbal tea was evaluated for phytoconstituents using Preliminary phytochemical analysis and DPPH assay for assessment of antioxidant activity. The combined tea of all the herbs (polyherbal tea) revealed high phenolic content (5,0300.01 mg CE/ L) and Low EC_{50}value (0.40±0.05) and High AE (2.50) as compared to single ingredient teas. The study has revealed the polyherbal tea of selected herbs had a high efficiency to show antioxidants health benefits associated with antioxidant need in the body.

Keywords: Polyherbal tea, Antioxidants, Infusion, Decoction.

1. INTRODUCTION

Antioxidants

Antioxidants are compounds that can prevent or postpone lipid peroxidation or other substances by preventing the start or growth of oxidative chain reactions, antioxidants. [1] The redox characteristics of phenolic compounds, which can be useful in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or dissolving peroxides, are primarily responsible for their antioxidant action. [2]

Herbal tea

Together with coffee and chocolate, tea is regarded as one of the top 3 drinks in the world. Chinese tea plants have been cultivated and utilised for over 3,000 years, as per ancient sources. Tea is generally recognised as a regular beverage in China and many other nations. Tea was being used as a medicinal or health product to cure and prevent a variety of illnesses since ancient times. Previous research has demonstrated the many advantages of tea, including its antioxidant, bactericidal, and anti-carcinogenic properties as well as its ability to control lipid metabolism. According to the degree of fermentation, tea may be broadly categorised into three types: green tea, oolong tea, and black tea. [3] The teas contain an enzyme called polyphenol oxidase, which is heat-labile. Green tea has so much more polyphenols compounds becauseteam warming during the brewing reduces the efficiency of this enzymes. [4] It has been demonstrated that oxidative stress is linked to a wide variety of clinical disorders. As a powerful antioxidant that scavenges oxidants and controls the activity of various metabolic enzymes in thebody, tea polyphenols can both prevent and treat illnesses. Because the hydrogen ion's capacity to attach to the phenolic hydroxyl structure is diminished and thus more liable to be split, the functional hydroxide ions neutralise the oxidants and other superoxide, salvaging the free radicals.[5]

Traditional herbs are promoted as a holistic therapy with the growing interest in living a healthierlife, and the vast majority of people utilise such herbs for their daily health care demands. [6] Making an infusion or decoction from the useful components of plants and herbs, such as blossoms, leaves, and roots, is now the most effective and widely used natural treatment. In the management of pain illnesses like cancer, digestive issues, and impaired glucose tolerance, herbal teas are commonly employed. [7]

Numerousresearchfindingssuggestt heroleofteainmediatingtheproperfunctioning ofthecardiovascularsystem, reducingbodymass,andevendecreasingtheriskofcancerandneurodegenerativediseases. [8] **Tea polyphenols**

The flavonoids molecules known as catechins, sometimes referred to as tea polyphenols or catechins, have a basic structure of -phenyl-benzopyran and make up between 18% and 36% of the dry mass of tea leaves. The four main categories of polyphenolic compounds are the most significant ones: Epigallocatechin-3-gallate (EGCG), epigallocatechin (3-gallate) (EC) epigallocatechin (EGC) and epigallocatechin (ECG). (Fig 1) [9]

These phytoconstituents antioxidant capabilities are mostly governed by the quantity and placement of hydroxyl groups in their molecular structures. As a result, the B and C rings of catechins have a significant ability to provide hydrogen, and the 2,3-double binding and open 4- oxo position in the C-ring help the ortho-dihydroxy catechol in the B-ring to delocalize its unpaired electron. [10]

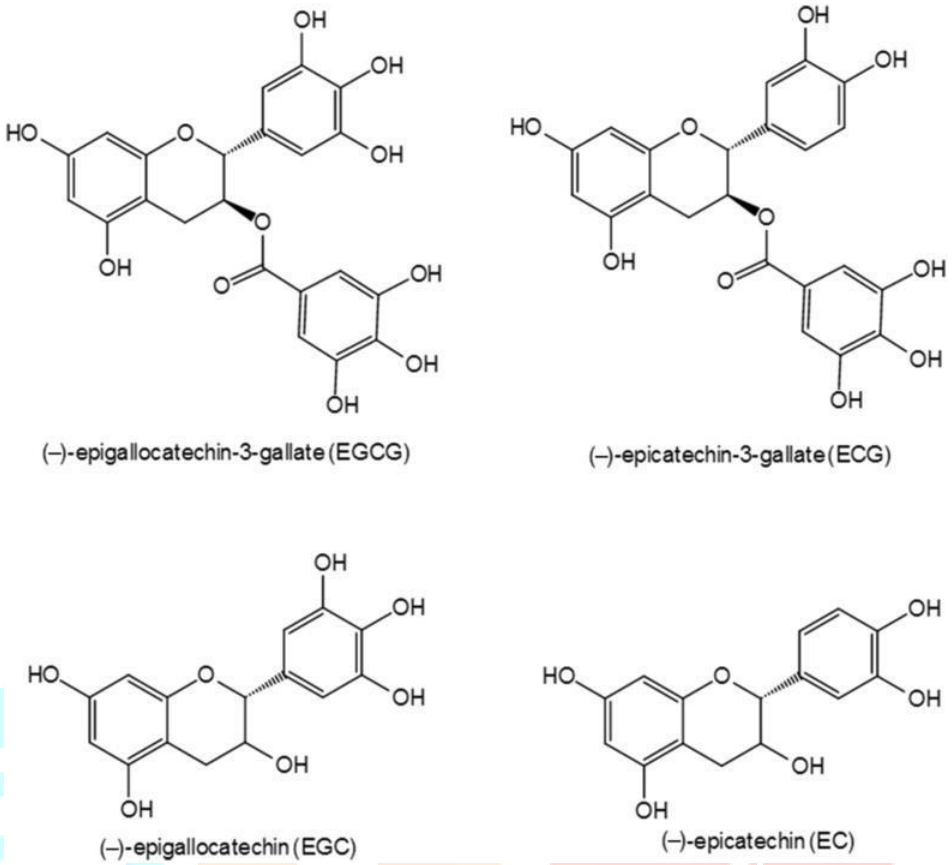

Fig 1. Catechins in the tea [9]

Protective capabilities of tea polyphenols against oxidants and oxidative stress

Reactive oxygen species (ROS), which are extremely active chemicals released throughoutenergy production and regular metabolic process, are strongly associated with both biologicaland pathological processes in mammals. The species mostly consist of hydrogen peroxide(H_2O_2), hydroxyl free radicals (OH), superoxide anion free radicals (O_2), and others. Wheneverthe body's protective mechanism and the buildup of ROS are out of balance, it results inoxidative stress and damages both cells and tissues, which leads to a number of disorders. [9] Catechins and other polyphenols have been revealed to increase protective antioxidants likecatalase, (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) along withthe reduction of malondialdehyde and TNF-α and caspase-3 in the liver[11,12]

2. PLANT PROFILE

1) Ashwgandha

Fig 2 Ashwagandha plant Common name: Indian ginseng

Botanical name: WithaniaSomnifera

Biological Source: Dried root of withaniasomnifera plant

Family: Solanaceae Geographical source:

Kingdom: Plantae

Chemical constituent: Steroidal lactone and alkaloids are withanolides, withaferine, witaferinA, withanone

Uses: Reducing stress and anxiety

Fig 3 Cinnamomum bark

2. Cinnamon

Commonname: CeyloncinnamonBotanicalname:Cinnamomumverum

Biological source: Cinnamon consistsof dried bark,freed from the outer cork andfrom theunderlyingparenchyma,fromshootgrowingonthecutstumpsofcinnamon

Family: Lauraceae

Geographicalsource: Srilanka,MalabarcoastofIndia,Jamaicaandbrazil.Kingdom:Plantae

3. Ginger root

Fig 4 Ginger root

Commonname:Ginger, Africanginger,JamaicangingerBotanicalname:Gingiberofficinale BiologicalSource:Gingerconsistsof therhizomesofzingiberofficinale,Roscoeanddriedinthesun.

Family: Zingiberaceae **Geographicalsource:**Jamaica,southindia,Africa,japan

Kingdom: Plantae

Chemicalconstituent: It consists ofgalanolactone,zingerone, and gingesulfonicacid.

Uses: Reduce blood cholesterol level, reduce the risk of cancer, reduce blood sugar.

4. Hibiscus:

Fig 5. Hibiscus

Commonname: Chinarose,Chinesehibiscus,shoeblackplant. Botanicalname: Hibiscusrosa-sinensis.

BiologicalSource:

It consistsofnumerousspeciesofherbs,shrub.andtrees.Family:Malvaceae

Geographicalsources: India,Bangladesh,Pakistan, Maldivesandalmostalloverworld.

Kingdom: Plantae **Chemicalconstituent:**HibiscussabdaritfaL.calyxareanthocyaninsandpolyphenols

.

Uses: Treatinglossofappetite,cold,heartandnervediseaseandswelling(inflammation).

5. Lemon grass

Fig 6. Lemon grass

Lemon grass is also known as barbed wire grass, silky heads, Cochin grass, Malabar grass, oilyheads, citronella grass, or fever grass.

Scientific name: Cymbopogon

Family: Poaceae

Kingdom: Plantae

Chemical constituent: Citral, Geraniol, Geranyl acetate, Myrcine.

Use: Stimulate the uterus and menstrual flow, and have antioxidant effects.antibacterial, against fever,

antidiabetic, lowers cholesterol

6. Stevia Leaf

Fig 7. Stevia Leaf

The plant Stevia rebaudiana, which is native to Paraguay and Brazil, produces leaves that are used to make stevia, a natural sweetener and sugar alternative. Steviol glycosides, namely stevioside and rebaudioside, are the active ingredients.

Chemical constituents : arepolyphenols, flavonoids, carotenoids, tannins, phenolic acids, chlorogenic acids, fatty acids, amino acids, proteins, and vitamins.

7. Clove

Fig 8. Clove flower buds

Cloves are the aromatic flower buds of a tree, Syzygiumaromaticum

Family: Myrtaceae

Scientific name: Syzygiumaromaticum

Family: Myrtaceae

Kingdom: Plantae

8. Moringa leaves:

Fig 9. Moringa leaves

Native to the Indian subcontinent, Moringa oleifera is a fast-growing, drought-resistant shrub of the

Family: Common names include "moringa," "drumstick tree," "horseradish tree,". Moringa tree leaves contain different polyphenols that mimic horseradish in extracts. Moringa oleifera belongs to Family Moringaceae

Kingdom : Plantae.

3. LITERATURE REVIEW

Büyükbalci A *et al* (2008) Evaluated In Vitro Antidiabetic Effects, Antioxidant Activities and Phenol Contents of Some Herbal Teas The effects of ten aqueous herbal tea extracts on the transit of glucose through the digestive organs were investigated in vitro. By looking at how extracts from herbal teas affected the scavenging of DPPH radicals and hydrogen peroxide, antioxidant properties of herbal teas were assessed. Green tea was found to have the highest peroxide inhibition value (65.50%) at a concentration of 250 l/ml.

Pal A *et al* (2011) studied the antioxidant activities of Withaniasomniferadunal root. Using assays for radical quenching activity, metal chelation operation, hydroxyl radical quenching activity, superoxide radical scavenging activity, and hydrogen peroxide scavenging activity, extracts were examined for their potential antioxidant properties. The most powerful fraction, according to the study's findings, was the methanolic extract, and there was a significantrelationship with its total phenolic content and potency.

Bernardo MA. *et al* (2015) studied effect of cinnamon tea on postprandial glucose concentrationCinnamon tea revealed a high free radical scavenging activity, resulting of its polyphenol concentration, according to a chemical investigation.

Jain P *et al* (2011) evaluated synergistic antioxidant potentials of polyherbal green tea.The herbal extracts combination's (Vitis vinifera, Phyllanthus emblica L, Punica granatum, Cinnamomum cassia, Ginkgo biloba L., and Camellia sinensis Linn.) antioxidant properties was on par with that of ascorbic acid in its purest form. Studies revealed that some plants had high concentrations of phenolics and flavonoids and that the polyherbal combination of these plants and green tea had the greatest antioxidant activity of all the separate extracts.

Atawodi S (2011)evaluatued and found "Antioxidant, hepatoprotective, and ameliorative activityagainst carbon tetrachloride-induced liver damage in rats at 5mg/kg were assessed for polyherbaltea (Tamarindus indica, Ginger officinalle, Khaya senegalensis, Nauclea latifolia, Moringa oleifera, Phyllantusamarus, Camellia sinensis, Anacardium occidentale, Mangifera indica, Aframomummelegueta and Morinda lucida)

4. AIM AND OBJECTIVE

Aim: - Preparation of polyherbal antioxidant tea in free radicals and protect our body.

Objective: -

1) To evaluate antioxidants in the tea which control the damaging effects of free radicals in thebody.

2) To reveal its use as a non-nutritive sweetener and herbal supplement.

5. PLAN OF WORK

I. Collection of all herbs

II. Procedure for the formulation of polyherbal tea

III. Phytochemical screening.

IV. Total phenolic contents

V. Test for flavonoids

VI. Test for phenols

VII. Test for alkaloid

VIII. Test for Steroids

IX. Antioxidant activity determination assay

X. Antioxidant Assay Using Ferric Reducing Antioxidant

1) DPPH assay

6. MATERIALS AND METHODMultiple Herbs for tea

Stevia leaves were purchased from Amazon online shopping platform, India Cinnamon, Clove, lemongrass, Ashwagandha and Hibiscus were received from Kamla Nehru College of pharmacy,Butibori, Nagpur Maharashtra, India.

Procedure for the formulation of polyherbal tea

Herbal teas were made using infusion or decoction techniques in accordance with the instructions on their labels (Table 1). Infusion: Teas were prepared using water; 100 ml of deionized hot water was added to a given quantity of tea, and the mixture was allowed to steep for 3 minutes without further heating. Decoction: A precise amount of tea was cooked in 100 ml of cold, deionized water for 15 minutes, followed by a 10-minute pause. All of the samples were run through Whatman No. 4 filter paper before being 55 °C vacuum-concentrated to a final volume of 50 ml. [6]

Table 1. Experimental herbal plants and methods for preparing their tea

Herbal plant samples	Used part (Powdered)	Concentration g/100 ml	Method used
Stevia leaves	Leaves	3.5	Decoction
Cinnamon	Bark	5	Infusion
Clove	Whole	2	Decoction
Lemon Grass	Leaves	3	Infusion
Ashwagandha	Roots	3	Decoction
Ginger	Rhizomes	3	Infusion
Hibiscus	Whole plant parts	4	Decoction
Combined polyherbal Tea	All herbs	5	Decoction

Fig.10Herbs in dried form

Phytochemical Estimation Preliminary phytochemical test [13]Test for alkaloids

A small portion of the solvent-free extract were stirred separately with 5ml of 1.5% v/v of hydrochloric acid and filtered. The filtrate

was tested with various test reagents for the presence of alkaloids. Mayer's reagent gave precipitate. Hager's test reagent gave yellow precipitate.Wagner's reagent gave reddish-brown precipitate. Dragendroff's reagent gave orange-brown precipitate.

Test for phenolic compounds and tannins

Small quantities of the extract were taken separately in water then warmed and filtered and test for the presence of phenolic compounds and tannins were carried out with the following reagents. With Ferricchloridesolution(5%) violetcolourationwasobserved, with Aceticacidsolution redcolourobserved, with potassiumdichromate redprecipitation wasobserved, with dilute Iodinesolution transientredcolourationwasobserved and with dilute potassiumpermagnatesolution decolourationwasobserved.

Test for flavonoids

With con. Sulphuric acid: To 2 ml of extracts, few drops of sulphuric acid were added. Yellow orange colour was observed. Additionally, **shinoda's test performed by adding few** dropsof conc.HCland 0.5gmagnesiumturnings to To dry powder of extract,5mlof 95% ethanol. Greencolourwasobserved.

Test for steroids

To 2 ml of extract, 2 ml of chloroform and 2ml conc. H_2SO_4 was added, chloroform layer appeared red and acid layer was observed with greenish yellow florescence. (Salkowski reaction)and to 2 ml extract with chloroform. 1-2 mlacetic anhydride and 2 drops conc. H_2SO_4 were added from the side of the testtube.First red then blueand finallygreencolouredwas observed (Libermann – Burchard reaction).

Total phenolic content

Using the Folin-Ciocalteu colorimetric technique, the quantity of total phenolic compounds was quantified utilising the normal (+)-Catechin and representing the outcomes as catechin equivalents (CE). 3 ml of liquid herbal tea samples (listed in Table 1) were mixed with 1 ml of 6M HCl. In contrast, after grinding, cinnamon and clove were weighed at 0.5 g, and 4.5 cc of 1.2M HCl were added. The tubes were vortexed and continuously swirled for 2 hours at 90 °C. After chilling, water was used to fill tube contents up to 10 ml.

100 mL of each sample were put into test tubes after being filtered through filter paper, and 1,900 mL of 10% Folin-reagent Ciocalteu's were then added. The tubes were combined, then let to sit at 50 °C for 20 minutes. With the use of a Pharmacia BiotecNovaspec II spectrophotometer, absorption at 760 nm was determined. The findings of each test were performed in triplicate and shown as means SD. [6]

Antioxidant activity determination assay Radical Scavenging

Activity against DPPH

Using the method outlined by Brand-Williams et al. and Parejo et al. the total antioxidant potential of the samples was calculated. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical test, the overall antioxidant capacity of the samples was assessed in terms of the capacity of tea preparations to scavenge free radicals. Following the preparation of a suitable diluted solution at 5 distinct strengths (0-0.2 ml sample/ml methanol), 0.1 ml of each fraction was mixedwith 3.9 ml of a 6.0105 M methanol solution of DPPH, and the mixture was vortexed. The reaction was permitted to plateau while being conducted at ambient temperature in the dark. [6]

7. RESULTS AND DISCUSSIONPreliminary test

The present study revealed significant outcomes of the tea revealing the presence of Phenolic andflavonoids responsible for the antioxidant potential. The preliminary test revealed positive outcome for the all the tests for phenolic and flavonoid estimation which showed presence of flavonoids and phenols.

Table no.2 Preliminary test results

TestFor	PEE	EAE	AE	ME
i)Alkaloids				
a)Mayersreagent	-	-	-	-
b)Hagersreagent	-	+	+	+
c)Wagnersreagent	-	-	-	-
d) Dragendorff reagent	-	+	+	-
ii) Test of flavonoid				
a) Shinoda test	-	+	+	+
b) sulphuric acid	-	+	+	+
iii) Steroid			-	-
a) Salkowski reaction		+	+	-
b) Libermann- Burchard reaction	-	-	-	-
iv) Test for tannins and phenols				
a) 5% FeCl3	-	+	-	-
b) Lead acetate test	-	+	+	+
C) Acetic acid	-	-	-	-

d) Pot. Dicromate	-	+	+	+
e) Pot. Permanganate reagent	-	+	+	+
V) Test for saponins Glycoside				
a) Foam test	-	-	-	-
VI) Test for carbohydrate				
a) Molish test	-	-	-	-

Total Phenol content

Per litre of herbal infusion, the amount of phenolic compounds was determined as milligram equivalents of catechin. (mg CE/L). Table 4 displays the total phenolic content of the investigated plants and drinks. In those herbal remedies, the total phenol content ranged from 70 mg CE/L for Ashwagandha, highest for combined polyherbal tea (5,0300.01 mg CE/ L). Lemon grass leaves to 4,070 mg CE/L for Lemon grass. Merely four tea herb varieties (stevia leaves, Hibiscus and Ginger) exhibited phenolic concentrations of more than 1,000 mg CE/L. Lemon grass infusion had the greatest phenol level (4,070 mg CE/L). Both the cinnamon stick and the clove were examined as solids, and their respective total phenol contents were 3.31 and 86.45 mgCE per gramme of material. Polyherbal tea revealed more phenolic content as compared to the other teas. (Table no. 2)

Table 3 The total phenolic content of the herbal tea samples

Herbal tea sample	**Total phenolic contents (mg CE/L or g tea)**
Stevia leaves	570±0.01 mg CE/ L
Cinnamon	3.31 ±0.03 mg CE/g
Clove	86.45 ±0.02 mg CE/ g
Lemon Grass	4,030±0.01 mg CE/ L
Ashwagandha	70±0.02 mg CE/ L
Ginger	200 ±0.02 mg CE/ L
Hibiscus	278 ±0.02 mg CE/ L
Combined polyherb. Tea	5,0300.01 mg CE/ L

Herbal tea samples and scavenging activities of samples on the DPPH radical

Utilizing the common free radical DPPH, it was determined if the materials could donate hydrogen. The quantity of sample required to lower the starting DPPH concentration. It is standard procedure to quantify the antioxidant activity using the (EC50) by 50% criteria. The power of antioxidants increases with decreasing EC50. Antiradical power or action is another factor (AE)

The radical activity increases as AE increases.

The range of EC50 values was 0.40 to 4.45 Combined polyherbal tea had the highest EC50 valueprobably followed by other single ingredient tea, while clove tea had the lowest ratings of all. (Table 3)

Table 4. Herbal tea samples and scavenging activities of samples on the DPPH radical

Herbal tea sample	**EC50**	**AE**
Stevia leaves	0.62±0.01a	1.61
Cinnamon	1.34±0.00e	0.07
Clove	4.95±0.05c	2.04
Lemon Grass	0.58±0.01d	1.72
Ashwagandha	0.99±0.09e	1.01
Ginger	2.98±0.09f	0.33
Hibiscus	3.93±0.01g	0.25
Combined polyherbal tea	0.40±0.05b	2.50

Every value represents the average and standard deviation of three independent assessments. Efficient concentration [EC50] [mg sample/mg DPPH.]: Amount of test sample required toreduce the starting DPPH, calculated as the concentration of stock solution supplied to thereaction mixture. [60 µM]

50% more concentration

Antiradical effectiveness (AE) = 1/EC50

Fig 11. Prepared polyherbal tea

8. CONCLUSION

Antioxidant sources are easily available but identifying, assessing, and ensuring particular herbs is important.The present study has revealed the polyherbal tea of selected herbs had a high efficiency to show antioxidants health benefits associated with antioxidant need in the body. The life of cells of the body can be increased with the help of polyherbal tea consumption. As the polyherbal tea can give greatest of the benefits in one cup it is needed to develop useful polyherbal tea preparations in the future to prevent the deadly lifestyle and other cellular disease.

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