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EVALUATION OF *INVITRO* ANTIOXIDANT ACTIVITY OF A NOVEL HERBAL TEA

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ABSTARCT:

Food nutrients having antioxidant potential could help to prevent many human oral disorders. Regular oral hygiene practices by chemical ingredients may lead to oral health damage. By this four plants namely *Syzgium aromaticum*, *Mentha piperita*, *Ocimum sanctum* linn ,*Azadirachta indica* herbal waters showing antioxidant potential individually, many oral disorders could be prevented . The aim of the present study was to study the antioxidant potential of the herbal waters of *Syzgium aromaticum*, *Mentha piperita*,*Ocimum sanctum* linn ,*Azadirachta indica* above plants by DPPH and phosphomolybdate *in-vitro* antioxidant screening methods. The results showed that IC50 value of *syzgium aromaticum* through DPPH assay method is 1.787842 gm/ml, and through phosphomolybdate assay method is 1.744722gm/ml ,*Mentha piperita* through DPPH assay method is 1.368432gm/ml, and through phosphomolybdate assay method is 1.782466 gm/ml, *Ocimum sanctum* linn through DPPH assay method is 2.45415gm/ml, and through phosphomolybdate assay method is 2.820216gm/ml, *Azadirachta indica* through DPPH assay method is 1.282051 gm/ml, and through phosphomolybdate assay method is 0.888889 gm/ml, and the herbal tea which was prepared in combinations of these herbal waters through DPPH assay method is 1.773375 gm/ml and through phosphomolybdate assay method is 1.934271 gm/ml as taking as ascorbic acid as standard. The IC 50 value of DPPH is 3.114504 gm/ml and in phosphomolybdate is 3.170846 gm/ml . As per our study this herbal tea of 6gm/100ml is having approximately half of antioxidant potential with ascorbic acid. Hence the consumption of herbal tea of 6gm/100ml may have the antioxidant potential as ascorbic acid and may help in the prevention of oral disorders.

Keywords: *Syzgium aromaticum*, *Mentha piperita*, *Ocimum sanctum* linn ,*Azadirachta indica* herbal DPPH assay, phosphomolybdate assay.

INTRODUCTION

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. The oxidants / free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either Oxygen derived (ROS) or Nitrogen derived (RNS). The most common reactive oxygen species include superoxide anion (O2), hydrogen peroxide (H2O2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxynitrite anion (ONOO), Nitrogen dioxide (NO2) and Dinitrogen trioxide (N2O3) . Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants with antioxidant property are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress[1-5].

OXIDATIVE STRESS:

"Oxidative stress" refers to an imbalance between oxidants and antioxidants that favours the Oxidants and may cause damage. Reactive oxygen species (ROS) are oxygen-derived molecules that are unstable and extremely reactive. These molecules include superoxide (O2), hydrogen peroxide (H2O2), and hydroxyl radical (OH). ROS are the most often seen free radicals in biological systems The generation of reactive oxygen species (ROS) is a crucial aspect of metabolism and is seen in a variety of physiological settings.

About half of adult patients suffer from oral cavity inflammatory disorders. In 10-15% of the global population, they are characterized by a decrease of the gingival tissue and, in the most extreme circumstances, a retraction of the underlying bone tissue. The majority of the time, extensive bacterial colonization of the gums is the first sign of these inflammatory diseases. By producing more reactive oxygen species (ROS) to disrupt harmful bacteria, these diseases set off the activation of host defensive mechanisms. Because ROS cannot discriminate between host tissues and pathogenic bacteria, which also possess antioxidant defences, they can harm the tissue of the organism that produced them as a "defense weapon" . Patients with periodontal disease who have compromised antioxidant defences are more vulnerable to ROS activity[6-8].

ANTIOXIDANTS:

Antioxidant "defense" system. The activation of antioxidant defense systems by the human body counteracts oxidative stress. Antioxidants neutralize excess reactive oxygen species (ROS) and function as scavengers of free radicals.

CLASSIFICATION OF ANTIOXIDANTS:

Antioxidants can be broadly classified into five categories which are explained below

• Primary antioxidants, also known as chain-breaking antioxidants, are substances primarily phenolic compounds that act as donors of hydrogen and electrons and break the free radical chains that result from lipid oxidation. Oxygen scavengers are compounds, such as ascorbic acid (vitamin C), that react with oxygen and so eliminate it in a closed system. Compounds classified as secondary antioxidants work by breaking down lipid hydro peroxides into stable end products.

• Enzymatic antioxidants are enzymes that work by eliminating highly oxidative species (like superoxide dismutase) or dissolved or head space oxygen (like glucose oxidase). Synergistic compounds known as chelating agents significantly increase the activity of phenolic antioxidants. The majority of these synergists, including amino acids, phospholipids like cephalin, and citric acid, show little to no antioxidant activity.

MODE OF ANTIOXIDANT ACTIVITY[9-15]

Traditional herbs are a major source of antioxidants that people consume on a daily basis. An suitable antioxidant balance can be found in a normal cell when the generation of oxygen species is enhanced or when the amounts of antioxidants are decreased. We refer to this condition as oxidative stress. Antioxidant defense mechanisms work to detoxify or scavenge free radicals such as reactive

oxygen species (ROS) and reactive nitrogen species (RNS) in order to counteract their damaging effects. The ability of the oxygen metabolites is influenced by antioxidants and compounds that can either reduce Reactive Oxygen Molecules (ROMs) or prevent their creation from forming in a strong reducing buffer. Thus, all reducing agents create defense mechanisms that keep the cell's amount of ROMs as low as feasible.

BENEFITS OF HERBAL TEA IN ORAL HEALTH[15-23]:

Herbal Tea:

Herbal tea is not at all like tea, even though it appears like tea and is prepared similarly. This is due to the fact that they are not derived from the *Camellia Sinensis* bush, which is the source of all tea. Tisanes, as herbal teas are officially termed, are infusions. The dried leaves, seeds, grasses, nuts, barks, fruits, flowers, and other botanical components that give herbal teas their flavour and health benefits are combined to make tiranes. Herbal teas don't contain caffeine, in contrast to other types of tea. They're also quite tasty and convenient to drink. Your herbal tea could have a single primary herbal component or a combination of herbal ingredients intended to create a particular goal, like rest, renewal, or alleviation from a certain ailment, among other things.

Uses of Herbal Tea:

• Another name for herbal teas, or "tisanes," are easy, affordable, caffeine- and drug-free ways to savor the flavor and health benefits of herbs and spices.
• You're giving your body some much-needed hydration by sipping herbal tea
• Reaching a more at ease and contented mental state.
• Encouraging heart health.
• Assisting with digestive and stomach issues.
• Offering the body purifying qualities.
• Encouraging vitality and health.
• Giving the nerve system nourishment.
• Boosting the immune system's power.
• Giving the body antioxidants.
• Energizing the body and increasing energy levels.
• Stress reduction.
• Aiding in preventing colds.
• Energizing the interior systems.
• Encouraging restful sleep.
• It tastes amazing and contains no caffeine.

Description of Clove:

Cloves consist of dried flower buds of Eugenia caryophyllus, (Family: Myrtaceae). It is indigenous to Amboyna and Penang Molucca islands. It is now cultivated chiefly in Zanzibar, Pemba, Madagascar, West Indies, Sri Lanka and India. In India, cloves are grown in Nilgiri, Tenkasi-hills and in Kanyakumari district of Tamil Nadu state. It is also cultivated in Kottayam and Quilon districts of Kerala. It should contain not less than 15 % (v/w) of clove oil. acid), resin, chromone and eugenin. The volatile oil contains eugenol (about 70 to 90 %), eugenol acette, methylamylketone, caryophyllenes and small quantities of esters and alcohols. Bio active compounds such as eugenol and caryophyllene contain anti-inflammatory activity. It has medicinal properties such as antimicrobial, anti inflammatory, anti-stress, antioxidant, antiviral

hepatoprotective, antinoceptive activities .Clove is also used as a dental analgesic, carminative, stimulant, flavouring agent, an aromatic and antiseptic[12-19].

Description of *Mentha piperita:*

Mentha piperita is popularly known as "pudina." It belongs to the family *Lamiaceae*. *Mentha piperita* is an aromatic herb, known for its essential oils. Pharmaceutical grade oil produced by distilling the fresh aerial parts of the plant at the beginning of the flowering cycle is standardized to contain no less than 44% menthol, 15%–30% menthone, and 5% esters, in addition to various terpenoids. Other compounds found in it are flavonoids (12%), polymerized polyphenols (19%), carotenes, tocopherols, betaine, and choline. The oil contains terpenoids such as α-pinene or β-pinene, α-phellandren, and also ester-connected with menthol or free acetic acid and isovaleric acid. Bio active compounds such as Methanol, Menthone, and peppermint oil contain anti-inflammatory activity. It has wide medicinal/pharmaceutical applications due to its antimicrobial, anti inflammatory, anti-emetic, anti spasmodic, carminative, diaphoretic and analgesic properties and it is used to treat bronchitis, anorexia, flatulence, colitis, nausea, migraines, headaches, anesthetic, myalgia and liver complaints[23-28].

Description of *Ocimum sanctum*

Tulsi consists of fresh and dried leaves of *Ocimum sanctum* Linn., belonging to family *Labiatae*. The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases in their day to day practice. In traditional system of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn. Bio active compounds such as caryophyllene, Ursolic acid, Rosmarinic acid and linalool contains anti-inflammatory activity. It has been recommended for the treatment of bronchitis, malaria, diarrhoea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The *O. sanctum* L. has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions. It has medicinal properties such as Anti- stress, antioxidant, hepatoprotective, immuno modulating, anti-inflammatory, antibacterial, antiviral, antifungal, antipyretic, antidiuretic, antidiabetic, antimalarial and hypolipidemic[12-22].

Description of *Azadirachta indica*

It consists of dried leaves of *Azadirachta Indica* belongs to family *Meliaceae*. Bio active compounds such as quercetin and beta-sisterol contains anti-inflammatory activity.v : Neem tree has numerous medicinal properties by virtue of its chemical compounds. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Apart from Azadirachtin , salannin, gedunin, azadirone, nimbin, nimbidine, nimbicidine, nimbinol, etc are other important liminoids of neem. : Neem tree has numerous medicinal properties by virtue of its chemical compounds. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Apart from Azadirachtin , salannin, gedunin, azadirone, nimbin, nimbidine, nimbicidine, nimbinol, etc are other important liminoids of neem.

Traditional Ayurvedic uses of neem include the treatment of fever, leprosy, malaria, ophthalmia and tuberculosis. Various folk remedies for neem include use as an anthelmintic, antifeedant, antiseptic, diuretic, emmenagogue, contraceptive, febrifuge, parasiticide, and insecticide[16-25].

MATERIALS AND METHODS[26-30]

Collection of plant materials

The leaves of *Syzygium aromaticum*, *Mentha piperita*, *Ocimum sanctum L.*,and *Azadirachta indica*, were collected from local market and washed with distilled water and shade dried for 6 to 7 days. Then the dried leaves were grinded into fine powder[15-20].

CHEMICALS:

DPPH (2,2,-Diphenyl -2-picryhydrazyl),Sodium phosphate ,0.6 m sulphuric acid ,Ammonium molybdate, L-Ascorbic acid, ethanol and distilled water.

Preparation of herbal tea:

The crushed powder of different plants (Tulasi, Clove, Mint and Neem) were weighed (2- 6g/100ml of H_2O) and mixed in equal ratio by weight. The mixture was boiled and filtered by using filter paper.

DPPH (2,2-DI PHENYL-2-PICRYL HYDRAZYL) SCAVENGING ACTIVITY[30]

Solution of DPPH is mixed with the substance which can donate a hydrogen atom which gives rise to reduce from with loss of violet colour. To evaluate free radical scavenging activity, Sample of different concentrations (2-6 gm in100ml) of 3ml of ethanol and 1ml of DPPH. Solution (0.1 mM dissolved in ethanol solvent) was prepared. Blank which contains 3.3 ml of ethanol and 0.5 ml of test solutions of different concentrations (2-6 gm in 100ml) Control which contains 3 ml of ethanol and 1 ml DPPH solution .After 90 minutes, absorbance was measured at 517 nm, % inhibition of DPPH activity is calculated by below formula % Inhibition = [Abssample – Abs blank] x 100/Abs control Where, Abs = absorbance .

PHOSPHOMOLYBDATE ASSAY[30]:

Ten millilitres each of 28 millimetre sodium phosphate, 0.6 millilitre sulphuric acid, and 4 millilitre ammonium molybdate solutions were combined to create the phosphomolybdate reagent. Samples of varying concentrations (2–6 gm in 100 ml) of extract solution were added to 30 ml of phosphomolybdate reagent. One milliliter of phosphomolybdate reagent was then added to each sample solution, and the mixture was incubated for 90 minutes at 95˚ in the dark. At 765 nm, the absorbance was determined using spectrophotometry. After 90 minutes, absorbance was measured at 765 nm for the control, which comprises 1 ml of ethanol and 1 m of phosphomolybdate reagent.

The percentage inhibition of the phosphomolybdate test was determined using the method below.

[Abs sample – Abs blank] x 100/Abs control = % Inhibition where absorbance (Abs) Equals.

RESULTS:

PHYSICAL AND ORGANOLEPTIC PROPERTIES

Sample	**Colour**	**Order**	**Taste**	**pH**
Clove Water	Dark brown	Aromatic	Spicy taste	4.4
Mint Water	Light green	Aromatic	Sweet	6.9
Tulasi Water	Green	Spicy Scent	Astringent	6.3
Neem Water	Light green	Pleasant	Bitter	7.8

PRELIMINARY PHYTOCHEMICAL STUDIES

Phytochemical Studies of Clove, Mint, Neem, Tulasi extract

Phytochemical test	**Clove**	**Mint**	**Tulasi**	**Neem**
Alkaloids	+	-	-	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	-	+
Steroids	+	+	+	+
Glycosides	+	-	+	-
Terpenoids	+	+	+	+
Phenols	-	+	-	+
Cardiac Glycosides	-	+	-	+
Carbohydrates	-	+	-	-
Proteins	-	+	-	-
Fats	-	-	-	-
Tannins	-	-	+	-
Resins	-	-	-	+
Terpens	-	-	-	-

Antioxidant activity of different samples of Clove, Mint, Tulasi, Neem waters

S.NO	**Amount of sample taken in 100ml of distilled water**	**powder of different plants**	**DPPH (2,2- Diphenyl1-1-picrylhydrazyl) Assay**		**Phosphomolybdate Assay**	
			Absorbance at 517nm	**% inhibition**	**Absorbance at 765nm**	**% inhibition**
1.	2g	Clove	0.331	46%	0.014	44.9%
		Mint	0.265	56.7%	0.010	52.38%
		Tulasi	0.331	46%	0.013	38.09%
		Neem	0.265	56.7%	0.013	58.09%
2.	3g	Clove	0.194	68%	0.003	68.8%
		Mint	0.208	66.06%	0.008	61.904%
		Tulasi	0.265	56.7%	0.011	53.6%

		Neem	0.190	69%	0.008	71.90%
3.	4g	Clove	0.002	77.5%	0.011	79.3%
		Mint	0.164	73.24%	0.005	76.19%
		Tulasi	0.208	66.06%	0.010	63.3%
		Neem	0.164	73.24%	0.006	74.428%
4.	5g	Clove	0.071	88.41%	0.008	86.2%
		Mint	0.105	82.87%	0.003	85.7%
		Tulasi	0.109	82.2%	0.007	86.6%
		Neem	0.066	89.23%	0.004	88.9%
5.	6g	Clove	0.006	90%	0.008	88.3%
		Mint	0.027	95.59%	0.041	95.23%
		Tulasi	0.025	95.92%	0.003	94.9%
		Neem	0.025	95.92%	0.002	93.6%
6.	Control		0.613		0.021	

Antioxidant activity of Herbal Tea (Clove, Mint, Tulasi and Neem):

S.NO	**Amount of sample taken from 100ml of distilled water**	**DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay**		**Phosphomolybdate Assay**	
		Absorbance at 517nm	**%Inhibition**	**Absorbance at 765nm**	**%Inhibition**
1.	2g	0.298	51.35%	0.0125	48.1625%
2.	3g	0.21425	64.94%	0.0075	64.051%
3.	4g	0.1345	72.51%	0.008	73.265%
4.	5g	0.0877	85.677%	0.00575	86.85%
5.	6g	0.02075	94.35%	0.0135	92.25%
6.	Control	0.613		0.021	

Antioxidant activity of Standard Ascorbic Acid:

S.NO	**Amount of sample taken from 100ml of distilled water**	**DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay**		**Phosphomolybdate Assay**	
		Absorbance	**%Inhibition**	**Absorbance at**	**%Inhibition**

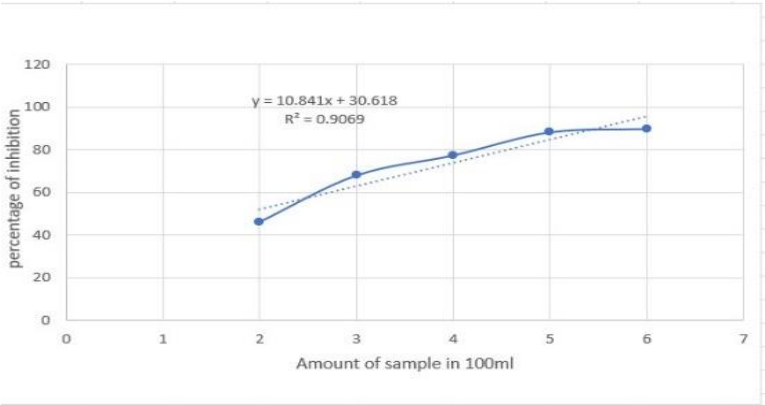
		at 517nm		**765nm**	
1.	2g	0.007	37.5%	0.016	36.8%
2.	3g	0.018	47.5%	0.012	44.8%
3.	4g	0.004	57.5%	0.0079	60.3%
4.	5g	0.002	77.5%	0.005	76%
5.	6g	0.073	88.09%	0.003	85%
6	Control	0.613		0.021	

IC50 Calculation:

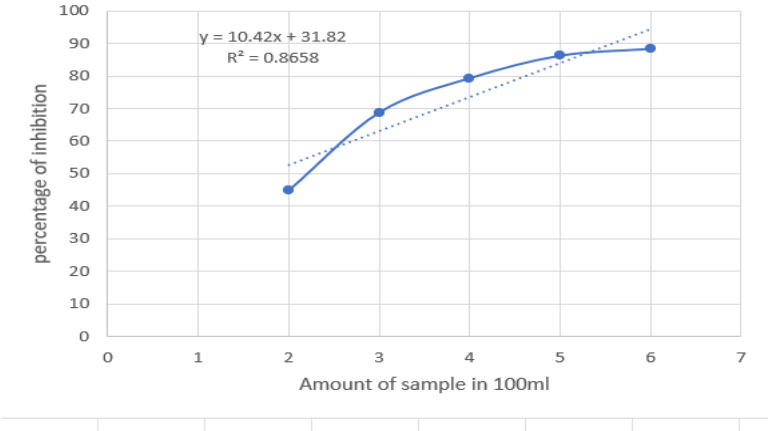
S.NO	**Sample**	**DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay**	**Phosphomolybdate Assay**
		IC50 Value (gm/100ml)	**IC50 Value (gm/100ml)**
1.	Clove water	1.787842	1.744722
2.	Tulasi water	2.454125	2.820216
3.	Neem water	1.282051	0.888889
4.	Mint water	1.368432	1.782466
5.	Herbal Tea (clove, tulasi, neem and mint)	1.773375	1.934271
6.	Ascorbic acid	3.114504	3.170846

Weight/ml of herbal waters					
Sample	**2g/100ml**	**3g/100ml**	**4g/100ml**	**5g/100ml**	**6g/100ml**
clove	1.01g/ml	1.0109g/ml	1.0129g/ml	1.01538g/ml	1.016180 g/ml
Neem	1.03406 g/ml	1.04184 g/ml	1.042587 g/ml	1.044070 g/ml	1.04592 g/ml

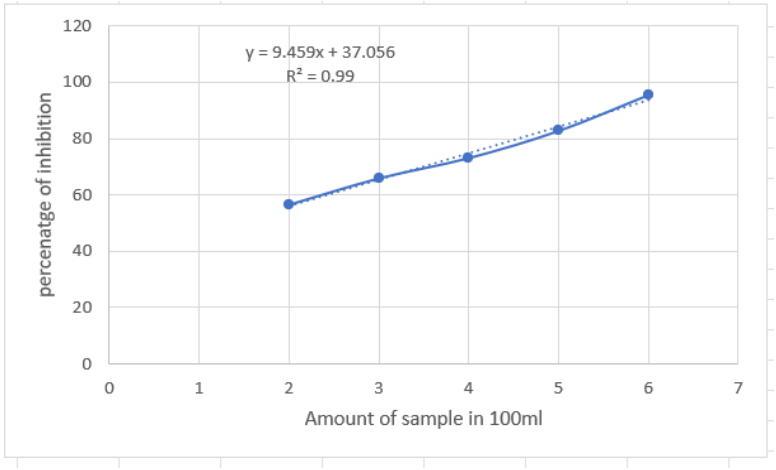
Mint	0.99401 g/ml	0.99588 g/ml	0.99961 g/ml	1.002972 g/ml	1.0044653 g/ml
Tulasi	0.999325 g/ml	1.001316 g/ml	1.00367 g/ml	1.00837 g/ml	1.02564 g/ml



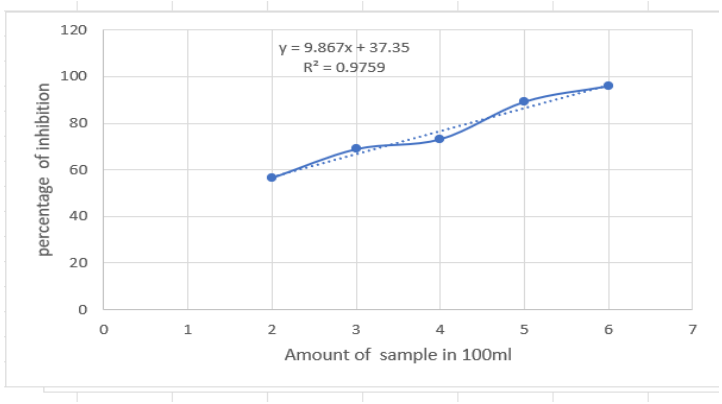
IC50 of clove water (DPPH Method)



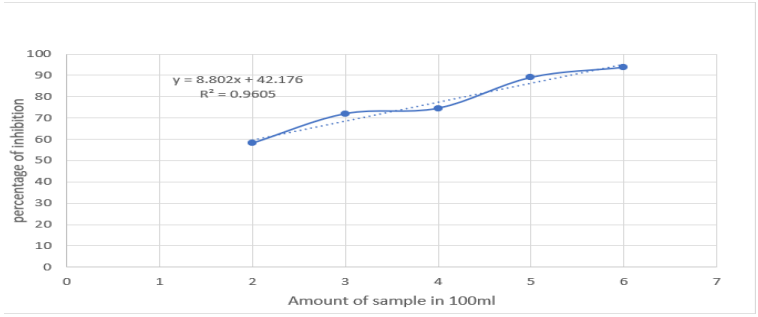
IC50 of clove water (phosphomolybdate Method)



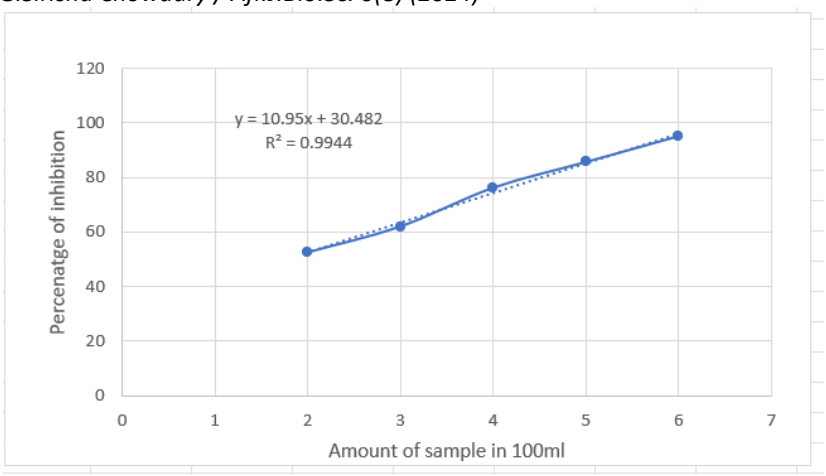
IC50 of Mint water (DPPH Method)



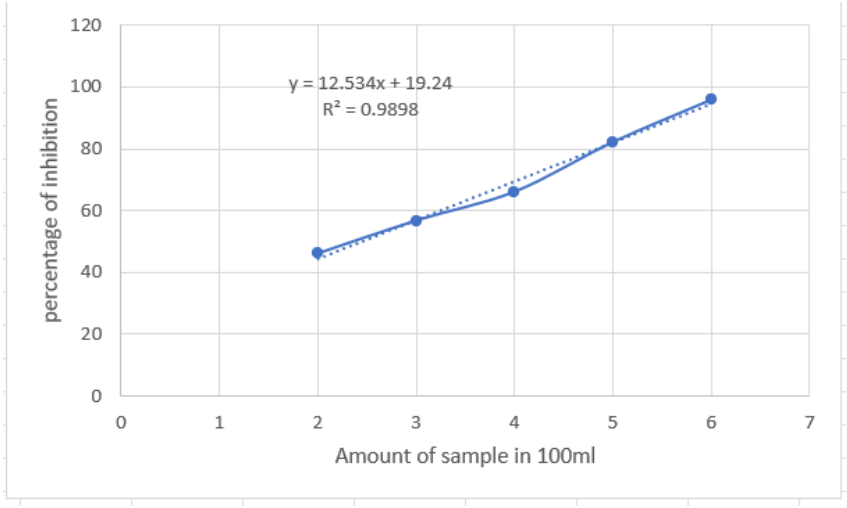
IC50 of Neem water (DPPH Method)



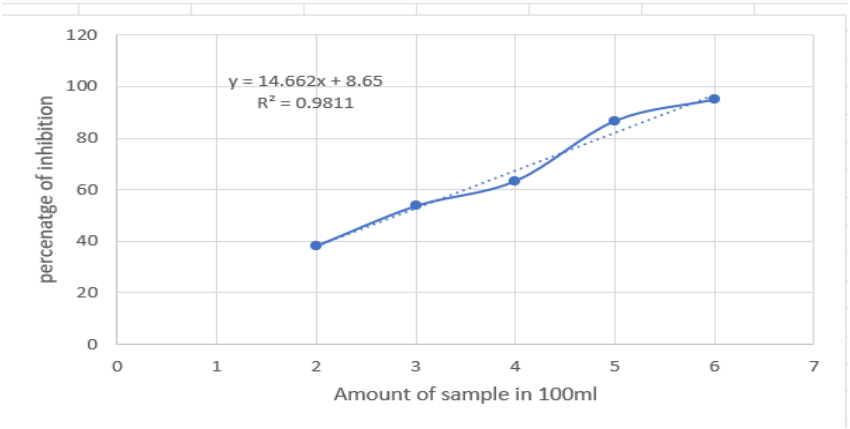
IC50 of Neem water (phosphomolybdate method)



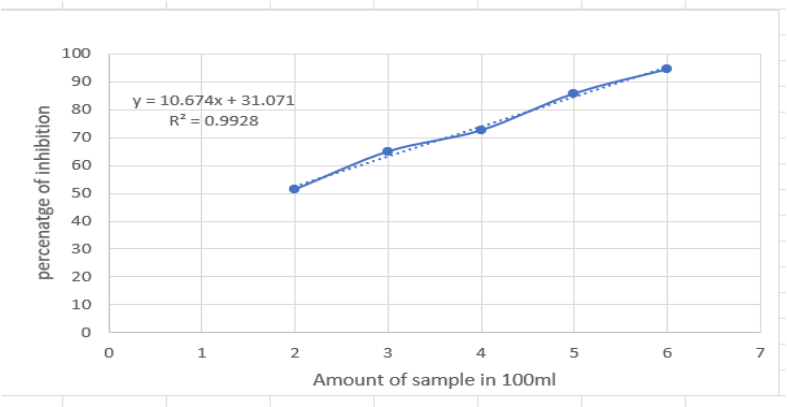
IC50 of Mint water (phosphomolybdatemethod)



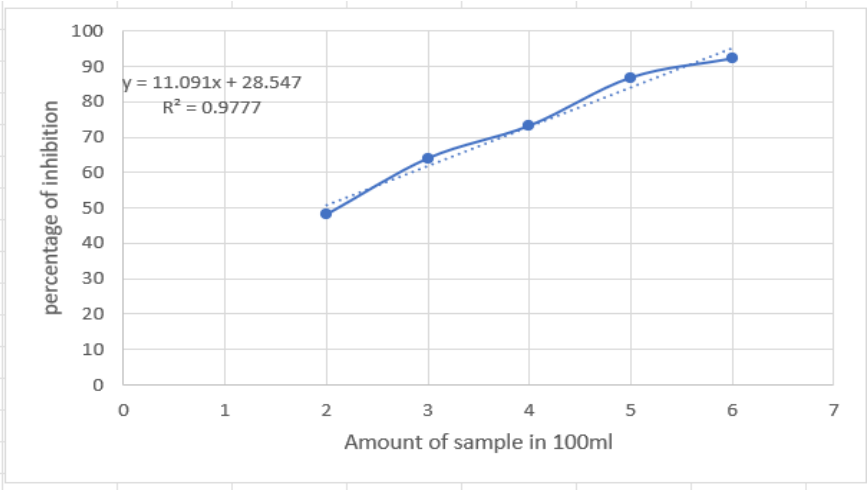
IC50 of Tulasi water (DPPHMethod)



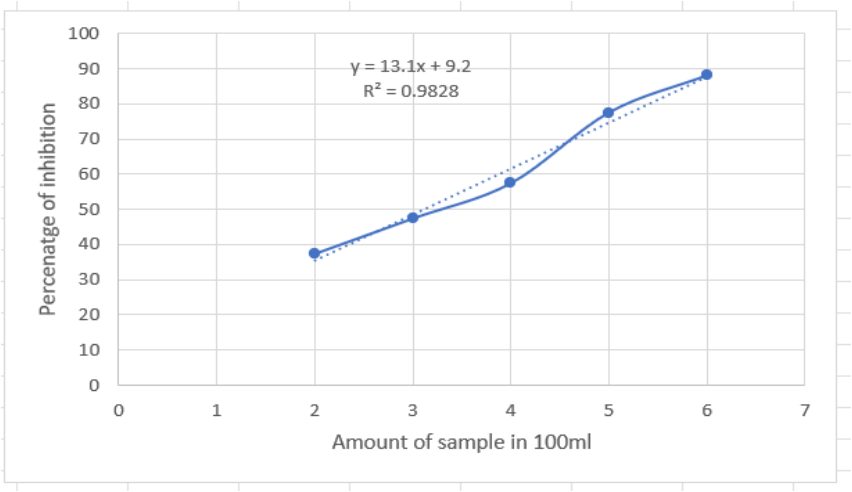
IC50 of Tulasi water (phosphomolybdate Method)



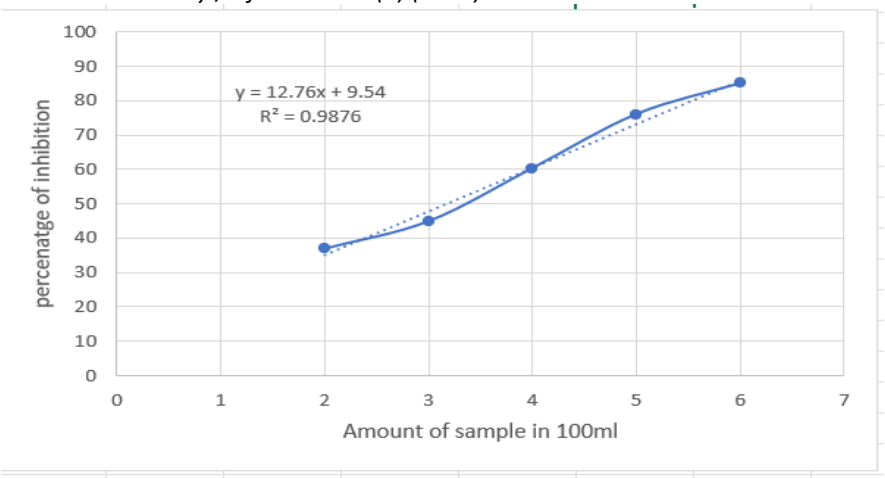
IC50 of Herbal tea (DPPH Method)



IC50 of Herbal tea (phosphomolybdateMethod)



IC50 of Ascorbic acid (DPPH Method)



IC50 of Ascorbic acid (phosphomolybdateMethod)

DISCUSSION

In this study, we evaluated the antioxidant activity of a water of *Syzygium aromaticum* by taking ascorbic acid as standard by using DPPH & phosphomolybdate assay method . In DPPH assay method the percentage of inhibition was estimated by using *Syzygium aromaticum* water. The water the amount of 2,3,4 ,5 and 6g/100ml concentrations Exhibited 46,58,77.5,88.4% 90 % inhibition respectively, where standard drug ascorbic acid at the same concentration exhibited 37.5, 47.5 ,57.5, 77.5 & 88.09% inhibition respectively .The IC5o (the concentration required to inhibit a radical formation by 50%) of water was found to be 1.787842g/100ml where as IC50 value of standard ascorbic acid was found to be 3.1145g/100ml

In phosphomolybdate assay the percentage inhibition by water of *Syzygium aromaticum* at different concentrations (2-6g/100ml) exhibited 44.9,68.8,79.3,86.2% 88.3% inhibition respectively whereas standard drug ascorbic acid at the same concentrations exhibited 36.8, 44.8, 60.3, 76, and 85% inhibition respectively the IC50 (g/100ml) value of Water of *Syzygium aromaticum* was found to be 1.744722 while that of ascorbic acid 3.1708.

In this study ,we evaluated the antioxidant activity of water of *Mentha piperata* by taking ascorbic acid as standard by using DPPH & phosphomolybdate assay method . In DPPH assay method the percentage of inhibition was estimated by using *Mentha piperata* water. The water at the amount of 2,3,4,5 and 6g/100ml concentrations exhibited 56.7, 66.06, 73.24, 82.87 & 95.59% inhibition respectively ,where standard drug ascorbic acid at the same concentration exhibited 37.5,47.5,57.5,77.5 & 88.09% inhibition respectively .The IC5o (the concentration required to inhibit a radical formation by 50%) of water was found to be 1.368432g/100ml where as IC50 value of standard ascorbic acid was found to be 3.1145g/100ml

In phosphomolybdate assay the percentage inhibition by water of *Mentha piperata* at different concentrations (2-6g/100ml) exhibited 52.38, 61.904, 76.19, 85.7 & 95.23%inhibition respectively whereas standard drug ascorbic acid at the same concentrations exhibited 36.8, 44.8, 60.3, 76,and 85% inhibition respectively IC50 (g/100ml)value of water of clove bud *Mentha piperata* was found to be 1.782466 while that of ascorbic acid 3.1708

In this study, we evaluated the antioxidant activity of water of by *Ociumum sanctum linn* taking ascorbic acid as standard by using DPPH & Phosphomolybdate assay method. In DPPH assay method the percentage of inhibition was estimated by using *Ociumum sanctum* linn water. The water at the amount of 2, 3 ,4, 5 and 6g/100ml concentrations exhibited 46, 56.7, 66.06, 82.2 & 95.92% inhibition respectively ,where standard drug ascorbic acid at the same concentration exhibited 37.5, 47.5, 57.5, 77.5 & 88.09% inhibition respectively .The IC5o (the concentration

required to inhibit a radical formation by 50%) of water was found to be 2.454g/100ml where as IC50 value of standard ascorbic acid was found to be 3.1145g/100ml

In phosphomolybdate assay the percentage inhibition by water of *Ocimum santum* linn at different concentrations (2-6g/100ml) exhibited 38.09, 53.6, 63.3, 86.6 and 94.9% inhibition respectively whereas standard drug ascorbic acid at the same concentrations exhibited 36.8, 44.8, 60.3, 76,and 85% inhibition respectively IC50 (g/100ml)value of water of *Ocimum sanctum* linn was found to be 2.8202 while that of ascorbic acid 3.1708

In this study, we evaluated the antioxidant activity of water of *Azadiracta indica* by taking ascorbic acid as standard by using DPPH & phosphomolybdate assay method . In DPPH assay method the percentage of inhibition was estimated by using *Azadiracta indica* water. The water at the amount of 2,3,4 ,5 and 6g/100ml concentrations exhibited 66.7, 69, 73.24, 89.23 & 95.92 % inhibition respectively ,where standard drug ascorbic acid at the same concentration exhibited 37.5, 47.5, 57.5, 77.5 & 88.09% inhibition respectively .The IC5o (the concentration required to inhibit a radical formation by 50%) of water was found to be 1.282051g/100ml where as IC50 value of standard ascorbic acid was found to be 3.1145g/100ml

In phosphomolybdate assay the percentage inhibition of water of *Azadiracta indica* at different concentrations (2-6g/100ml) exhibited 58.09, 71.90, 74.428, 88.9 & 93.6 %inhibition respectively whereas standard drug ascorbic acid at the same concentrations exhibited 36.8, 44.8, 60.3, 76 and 85% inhibition respectively IC50 (g/100ml) value of water of *Azadiracta indica* water was found to be 0.8888 while that of ascorbic acid 3.1708.

In this study, we evaluated the antioxidant activity of water of herbal tea (*Syzygium aromaticum, Mentha piperata, Ocimum santum* linn *and Azadiracta indica*) by taking ascorbic acid as standard by using DPPH & phosphomolybdate methods . In DPPH assay method the percentage of inhibition was estimated by using *Syzygium aromaticum, Mentha piperata, Ocimum santum linn and Azadiracta indica* waters. The water at the amount of 2, 3, 4,5and 6g/100ml concentrations exhibited 51.35, 64.94, 72.51, 85.677, 94.35% inhibition respectively,where standard drug ascorbic acid at the same concentration exhibited 37.5, 47.5, 57.5, 77.5 & 88.09% inhibition respectively .The IC5o (the concentration required to inhibit a radical formation by 50%) of waters was found to be 1.773375g/100ml whereas IC50 value of standard ascorbic acid was found to be 3.1145g/100ml

In phosphomolybdate assay the percentage inhibition by water of *Syzygium aromaticum, Mentha piperata, Ocimum santum* linn *and Azadiracta indica*, at different concentrations (2-6g/100ml) exhibited 48.1625, 64.051, 73.265, 86.85, 92.25 %inhibition respectively whereas standard drug ascorbic acid at the same concentrations exhibited 36.8, 44.8, 60.3,76,and 85% inhibition respectively IC50 (g/100ml)value of waters of *Syzygium aromaticum, Mentha piperata, Ocimum santum linn and Azadiracta indica*was found to be 1.934271 while that of ascorbic acid 3.1708.

CONCLUSION

In the present study , the novel herbal tea has been proved with antioxidant property by DPPH & Phosphomolybdate assay methods. Which contributes in the maintenance of oral health. As per our study this herbal tea of 6gm/100ml is having approximately half of antioxidant potential with ascorbic acid. Hence the consumption of herbal tea of 6gm/100ml may have the antioxidant potential as ascorbic acid and may help in the prevention of oral disorders.

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