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ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF *Ocimum tenuiflorum* LEAF AND *Zingiber officinale* STEM EXTRACTS

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ABSTRACT

Objective: The aim of the present investigation was to extract various phytoconstituents from *Ocimum tenuiflorum* leaf and *Zingiber officinale* stem using ethanol, chloroform, and n-butanol as solvent and characterized for biomedical applications.

Material and methods: Obtained extracts were used for phytochemical screening and characterized in terms of ash value determination, TLC and FTIR spectral analysis. Various concentrations of extracts viz. 1, 2, 3, 4 and 5 µg/ml were prepared and evaluated for free radical scavenging potential against DPPH. Ascorbic acid was used as standard to compare free radical scavenging activity. Antimicrobial efficacy of extracts was also determined against *Escherichia coli* and *Bacillus subtilis*.

Result and discussion: Results showed that chloroform extract of *Zingiber officinale* have best antioxidant activity among all the extracts. Ethanolic extract of ginger showed significantly higher efficacy against *B. Subtilis* while ethanolic extract of *Ocimum tenuiflorum* has higher antimicrobial efficacy against *E. coli*. Extracts were also good antioxidant activity.

Conclusion: It can be concluded from the findings of the result that *Ocimum tenuiflorum* and *Zingiber officinale* have significant antioxidant and antimicrobial effect and can be used in pharmaceutical, food and cosmceuticals industry.

Keywords: *Ocimum tenuiflorum* leaf; *Zingiber officinale* stem; antioxidants; free radical, herbal extract

INTRODUCTION

Zingiber officinale and *Ocimum sanctum Linn* has been used as a Chinese and Indian medicine for over 25 centuries. Fresh *Zingiber officinale* contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates *Ocimum sanctum Linn* have yellow colored and pleasant volatile oil 0.1- 0.9 %. The active components were reported to stimulate digestion, absorption, relieve constipation and flatulence by increasing muscular activity in the digestive. **David *et al*** evaluated the herbal extracts come in a lot of forms, they have been familiar feature. Herbal extracts represents in nature occurring photochemical activity that have been removed from the inert structural material of the plant **[1]**. **Kharbach *et al*** to use as an herbal extracts of plants materials (raw materials) for medicinal, formulations, cosmetics, fragrances, nutrition, and food additives implies also the need for sophisticated analytical techniques and methods to assesstheire quality **[2]**. **Farzaneh *et al*** used the term Herbal extracts refers to deferent morphological plants parts of, this include flowers, leaves, buds, stems, branches, rhizomes, seed, fruits and roots. Herbal extracts are substances from the plant using different solvents-some combination of water, alcohol, chemicals, or other liquid that works to draw out beneficial plant components. The process of herbal extraction is usually designed to maximize a certain portion of the original chemical compounds found in the plants, many of which have a therapeutic action **[3]**. **Kuttan *et al*** used to alcoholic extracts of ginger is more cytotoxic than aqueous herbal extracts in cultured Dalton's lymphoma as cites tumor cells, human lymphocytes and Chinese Hamster Ovary cells and Vero cells **[4]**. **Steinberg *et al*** used a number of studies have investigated the activity of plant extracts and products against specific oral pathogens, while others have focused on the ability of the products to inhibit the formation of dental biofilms by reducing the adhesion of microbial pathogens to the tooth surface, which is a primary event in the formation of dental plaque and the progression to tooth decay and periodontal diseases **[5]**. **Jude *et al*** to investigate the herbal extracts and its fraction dose – dependently reduced parasitaemia induced by chloroquine-sensitivity plasmodium berqhei infection in prophylactic, suppressive and curative models in mice **[6]**. **Devaraj *et al*** evaluating the present study were aimed at the antiulcer activity of the herbal extracts

formulation containing leaf of *Moringa oleifera*, *Raphinus sativus*, and *Amaranthus tricolor* was found to possess antiulcer properties in three experimental animal models of gastric ulcers, and these findings suggest that the significant gastroprotective activity could be mediated by its antioxidant activity **[7]**. **Fatima *et al*** to evaluate herbal extracts, as a whole or part, have been used for various ailments of the skin, hair, and dental care for overall appearance. Cosmetics alone are not sufficient to take care of skin and others body parts, it requires association of active ingredients to check the damage and ageing of the skin **[8]**. **Oh *et al*** used a various leafy herbal tea extracts compared antioxidant and antimicrobial activity of (water, ethanol). The material included: rooibos, green tea, black tea, rosemary, lemongrass, mulberry leaf, bamboo, leaf, lotus leaf, peppermint, persimmon leaf, and mate tea. They concluded that ethanol extracts of green tea, rosemary, mate, and persimmon leaf teas exhibited considerable antioxidant potential **[9]**. **Fotakis *et al*** to evaluate the herbal extracts were compared antioxidant potential and metabolomics in infusions and decoctions. Infusion is made when herb is added to already boiling water and decoctions is made up with cold water and then boiled. From carried out analyzes, it was concluded that both metabolic and antioxidant profiles have higher values for infusions **[10]**. **Mittal *et al*** used and identified *Ocimum tenuiflorum* is an Ayurvedic herbal plants widely used in therapeutic herbal and true tea blends, may be called tulasi, holy basil, "The Incomparable One," "Elixir of Life," or "Queen of the Herbs." Native to India and cultivated throughout Southeast Asia, it's considered a foundational herb that, combined with other adaptogenic herbs, can help the body withstand many forms of stress **[11]**. In a study of herbal extracts of *Ocimum sanctum Linn* and *Zingiber officinale* were found an antioxidant activity, antimicrobial activity, and analysis of TLC and IR spectroscopy. Different herbal extracts were used as an antioxidants activity **[12]**, antimicrobial activity **[13]**, and antdiabetic activity **[14]**, Antiulcer activity **[15]** Anti-inflammatory, analgesic and antipyretic activity **[16]**, analgesic, antipyretic and anti-inflammatory activity **[17]**, Anticancer Activity **[18] [19]**, Wound Healing Activity **[20]**.

Aim of work: The aim of work to investigate the extract of various phytoconstituents from *Ocimum tenuiflorum* leaf and *Zingiber officinale* stem using ethanol, chloroform, and n-butanol as solvent and characterized for biomedical applications.

MATERIALS AND METHODS

Materials and methods:

Materials: The ginger rhizome was purchased from the local market of Greater Noida U.P., India. All other chemicals and reagents used in the study were AR grade and were purchased from CDH, New Delhi. Tulsi plants were collected from a local area of Omicron 1A Greater Noida U.P.India, (28° 35' N, 77° 12' E).

Methods

Collection of (*Ocimum sanctum Linn)* leaves*:* Fresh plant leaves of *Ocimum sanctum L.* were collected from a local area of omicron 1A Greater Noida U.P. (28° 35' N, 77° 12' E). The leaves were washed thoroughly under tap water followed by sterile distilled water. Then leaves were dried under shaded condition at room temperature. Sampling of *Ocimum sanctum L.* was planted in the month of March. Middle aged fresh leaves of *Ocimum sanctum L.* were plucked during the month of September- October in the morning between 9-10 a.m. (IST) when dew was less and temperature was also not so high.

Collection of ginger stems (*Zingiber officinale*): Fresh ginger stem (*Zingiber officinale*) were procured from local market. The stem were washed with distilled water and dried in oven at 40 °C, about 5 to 10 min.

Extraction of plant material: Dry ginger was crushed to a coarse powder and extracted with 95 % ethanol by simple maceration process. Solvent was evaporated at room temperature. The residue obtained was dried. As well as the dried leaves material (in 20 gm) of *Ocimum sanctum L.* was extracted with 200 ml volumes of solvents, Ethanol, chloroform and n-butanol, separately at room temperature, in succession about 24 hours. The organic solvent was separated **[21]**.

Tulsi and Ginger Extract physicochemical characterization (TE and GE):

Identification test for proteins, carbohydrates and tannins: Chemical characterization was done by using aqueous GE and TE. Test for proteins, alkaloids, carbohydrate, tannins, glycosides and amino acid was performed according to standard procedure **[22]**.

Test for carbohydrates

Molisch'test: Extract solution (1 %) was prepared in distilled water. About 2 drop of α-naphtha in alcohol was added and mixed well followed by addition of concentrated sulphuric acid in drop wise manner form the sides. A Purple ring was observed at the junction of two liquids indicates the presence of carbohydrates.

Test for reducing sugars: Fehling's test 1 ml from each of Fehling's solution A and B

were added to the 1 % of Extract solution, mixed and heated from 5 min to 10 min on water bath. Initially a yellow precipitate followed by brick red color precipitate was formed which indicates the presence of reducing sugars.

Benedict's test: Equal volumes of Benedict's regent and TPS solution were heated over boiling water bath for 5 min. A Green/ yellow or red color was observed indication presence of reducing sugars.

Test for lipids: Sudan red III test: Sudan red III reagent was added in to 1% of prepared sample solution. No red color was observed indicates the absence of lipids in polymer.

Test for tannins: About 2 ml of aqueous extract was stirred with 5 ml of distilled water and few drops of ferric chloride ($FeCl_3$) solution were added. Formation of green precipitate was identification of tammins.

Methanolic extract of Zingiber officinale with 10 (%) lead acetate solutions: No white predicate was formed.

Methanolic extract of Ocimum sanctum L. with 10 (%) lead acetate solutions: No white predicate was formed.

Methanolic extract of Zingiber officinale with 5 (%) 0f ferric chloride solutions: No deep blue color was observed.

Methanolic extract of Ocimum sanctum L. 5 (%) 0f ferric chloride solutions: No deep blue color was observed.

Methanolic extract of Zingiber officinale with 10(%) of potassium dichromate solution: No red color predicate was formed. It indicates that tannin was absent.

Methanolic extract of Ocimum sanctum L. with 10(%) of potassium dichromate solution: No red color predicate was formed. It indicates that tannin was absent.

Test for Saponins: About 5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and heated over boiling water bath for 5 min. The formation of stable foam was observed as an indication of the presence of saponins.

Test for aminoacids: Ninhydrin test: Extract of *Ocimum sanctum L.* was heated with 5% of ninhydrin reagent in boiling water bath for 10 mints. Absence of purple/bluish color indicates the absence of amino acid in ocimum tenuiflorum.

Test of Alkaloid: Dragendroff's, Mayer's, Hager's, Wagner's test were carried out to identify the presence of alkaloid in GE and TE.

Phlobatannins test: About 2 ml of aqueous *Ocimum sanctum L.* extract was added to 2 ml of 1(%) HCl solution and boiling water bath for 10 mints. Deposition of red

precipitate was taken as evidence for the presence of phlobatannins.

Flavonoids test: About 1 ml of Extract solution added 1ml of 10 (%) lead acetate solutions. The formation of a yellow precipitate was taken as a positive test for flavonoids **[23]**.

Estimation and Validation of λ_{max} of extracts (TE and GE): The method was validated according to ICH Q2 (R1) guidelines for validation of analytical procedures using phosphate buffer solution (PBS) pH 7.4, phosphate buffer solution (PBS) pH 6.8, and buffer solution of 0.1 N HCl. Wavelength of maximum absorption (λmax) was determined by scanning 10 µg/ml solution of using UV- Visible double beam spectrophotometer (Shimadzu UV-800) from 200-800 nm for six days in three times (9:30 am, 2:30 pm and 4:30 pm) **[24]**.

Determination of Ash value:

a. **Determination of total ash:** Total ash was determined by weighing 0.078mg of the air dried crude drug in the silica dish and incinerated at a temperature not exceeding 450 °C then was cooled and weighed **[25]**. Total ash was determined using following equation 1

$$\text{Total Ash value (\%)} = \frac{\text{wt.of total ash}}{\text{Wt.of crude drug taken}} \text{X } 100$$

....equation 1

b. **Determination of acid insoluble ash:** The ash obtained as discussed in previous process (a) was boiled with 25 ml of 2 M HCl for 5 min. The insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a desecrator and weighed. Percentage of acid insoluble ash was calculated (equation.2) with reference to the air dried drug.

$$\text{Acid insoluble ash value (\%)} = \frac{\text{Wt.of acid insoluble ash}}{\text{Wt.of crude drug taken}} \text{ X 100 ....equation 2}$$

c. **Determination of water soluble ash:** (a) was boiled with 25 ml of water for 5 min. The insoluble matter was collected on ash–less filter paper and was washed with hot water, ignited for 15 min at a temperature no exceeding 450 °C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water soluble ash. Percentage of water soluble ash (equation 3) was calculated with reference to the air dried drug.

$$\text{Water soluble ash value (\%)} = \frac{\text{Wt.of acid insoluble ash}}{\text{Wt.of crude drug taken}} \text{ X 100 .....equation 3}$$

Determination of Melting Point: Melting Point of Plants Extracts (TE and GE) was examined by open capillary method. Plants

extracts was taken in glass capillary whose one end was closed by flame. Capillary containing extracts was kept inside the melting point apparatuses. Purity of sample of extracts can be easily identified by melting points **[26]**.

FTIR spectral analysis of extracts (TE and GE): The FTIR (Fourier Transform Infrared Spectrophotometer) analysis shows the presence of different functional group (chemical bonds) such as carboxylic acids, alcohols, phenols, aliphatic, amines, aromatics, and alkenes groups in the various extracts. The FTIR spectra are recorded in KBr by sophisticated computer controlled FTIR Perkin Elmer Spectrometer **[27]**. The extracts were used for FTIR analysis. Each sample of extract was loaded in FTIR spectrophotometer (Shimadzu) with a scan spectral range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Thin layer Chromatography analysis (TLC): TLC study was performed characterize GE and TE. GE and TE were dissolved in Chloroform, n-Butanol and Ethanol. Pre-coated silica gel plates (Merck 60 F 254) were used as stationary phase. Different solvent system Chloroform, n-Butanol, Benzene, n-hexane, and 1:1 Chloroform: n-butanol, 1:1 n-butanol: benzene, water, 1:1 water: n-Butanol was used as mobile phase **[28]**.

Antioxidant activity: Herbal Plant Extract has been used as antioxidant in pharmaceutical, cosmetic and Food industry.

$DPPH^{I}$ radical scavenging properties: DPPH radical scavenging assay is a standard method to evaluate antioxidant potential i.e. phytochemicals. Scavenging ability of phytochemical was compared with standard antioxidant (ascorbic acid). To evaluate antioxidant activity of 0.1 mM solution of DPPH was prepared by adding 1.9 mg DPPH in 100 ml volumetric flask and methanol compound were used as a solvent. Sample solution were prepared in various concentrations *viz.*01, 02, 03, 04 and 05 µg/ml same concentration of ascorbic acid was also prepared. Each sample solution (1ml) was added with same volume of DPPH solution, mixed vigorously and kept aside in dark place for 30 min. Absorbance were measured using UV spectrophotometer. Scavenging (%) is inhibition (%) was calculated using following equation 4. Test was performed in triplicate and the graph was plotted with the average of three observations with standard deviation **[29]**.

% inhibition (or % scavenging)

$$= \frac{(\text{Absorbance of control sample} - \text{Absorbance of test sample})}{\text{Absorbance of control sample}} \times 100$$

….equation 4

H_2O_2 radical scavenging activity: In this study hydrogen peroxide was used to form hydroxyl radicals. Standard and sample solution was prepared as above and concentrations of hydroxyl radicals were evaluated at 230 nm. (%) inhibition (or % scavenging) activity was calculated by using equation 4. Tests were performed in triplicate and the graph was plotted with the average of three observations with standard deviation **[30]**.

Antimicrobial efficacies of plant extract: Antimicrobial activity of plant extract was evaluated and compared using disc diffusion method. Test microorganism was obtained from the Department of Medical Lab Technology, School of Medical and Allied Sciences, Galgotias University, India, and comprised the gram-negative bacteria *Escherichia coli* and *Bacillus subtilis*. The microorganism was initially cultured on sterilized nutritive agar medium both *Escherichia coli* and *Bacillus subtilis* were cultured for 24 h at 30 °C. Extracts were dissolved and diluted in double distilled water to prepare 10 mg/ml solutions. Solutions were poured onto 5-mm discs and incubated for the next 24 h. After incubation, inhibition zone was measured in millimeters and antimicrobial activity of extracts was compared **[31]**.

RESULTS AND DISCUSSIONS

Physico-chemical Characteristics: *O. sanctum L.* is used in the treatment of various disease conditions. The standardization of a crude drug is an integral part of establishing its correct identity. The present study is associated to pharmacognostical, physical constants and preliminary phytochemical screening of *Ocimum sanctum* L. and *Zingiber officinale.* Phytochemical study is also useful to isolate the pharmacologically active principles in the drug. Results of the study were shown in the **Table 1**.

Estimation and Validation of λ max of extracts (TE and GE): Extracts were characterized in term of maximum absorbance (λmax). The results of the study were show in **Table 2**.

Determination of Ash value: Ash value is useful in determination and purity of sample and also these values are important qualities standards. The total ash value, acid insoluble ash, water soluble ash was found to be 20.512 %, 0.141%, and 17.05%, respectively for ethanolic extracts of O.S. lave and 4.8 %, 1.20 %, and 1.79 %, respectively for ethanolic extracts of Z.O. rhizome.

Determination of Melting Point: Melting Point of TE extracts was founds more than 255 °C. These relationships indicated a true melting point of 134.4 °C and a USP melting range of 135.2 ± 136.4 °C which is in reasonable agreement with the values that we derived above from the data supplied with the phenacetin reference compound, namely 134.6 ± 0.13 °C and 135.5 ± 136.1 °C respectively.

FTIR analysis of extracts (TE and GE): FTIR spectrum is used to identify the functional group of the active components based on the peak value in the region of infra red radiation. FTIR spectrum confirmed the presence of alcohol, phenol, alkenes, alkyl halide, amino acids, carboxylic acid, and aromatic, amines in the (TE and GE) extracts **[15]**. The data on the peak values and the probable functional groups were represented in **Table 1** and the FTIR spectrum profile was illustrated in the **Figure 3-7**.

Thin layer Chromatography analysis: Initially TLC study was performed using different solvent systems viz. in chloroform, n-butanol and ethanol solvent and acts as sample. No spots were observed when plates were kept in iodine chamber as spraying agent. Further spraying agent was changed to 40 % perchloric acid but results were same (no spots were found). Furthermore choloroform, n-butanol and benzeen were used as mobile phase in different ratios with 10 % Chloroform with 10 % n-Butanol, 10 % Benzene with 10 % n-hexane, and 1:1 Chloroform: n-butanol, 1:1 n-butanol: benzene, water, 1:1 water: n-butanol was used as spraying agent. TLC analysis easily predicts that gum contains polar compound, as spots were not observed with non-polar mobile phase. As polarity of mobile phase increased spots were observed with suitable spraying agent. It can be concluded from study that leaf extracts sample contains polar compounds which moves with polar mobile phase only. TLC characteristics of Plant extract were tabulated in **Table 8**.

Antioxidant activity of extract using DPPH Free radical scavenging activity model: DPPH has been widely used to evaluate antioxidant activity of various materials obtained from plant and microbial sources. DPPH is a chemical that create free radicals and these radicals are further scavenged by antioxidants. This experiment easily shows that extract has compound that easily scavenged free radicals by donating free hydrogen (proton) to free radical in order to remove old electron, which is responsible for the radical's reactivity. Hydrogen donating properties of polysaccharide can be supported by IR spectra analysis (show

presence of –OH groups). **Figure 6** show that as the concentration of extract increases scavenging properties of extract also increases. The effective concentration (EC50) sufficient to scavenge 50% of free radical was calculated and found to be 4.55 µg/ml ± 0.99 and 29µg/ml ± 1.21 for ascorbic acid and extract, respectively.

H_2O_2 radical scavenging activity: Figure 7 show that extract has hydroxyl group activity in concentration dependant manner. As the concentration of extract increases hydroxyl radical neutralizing power of extract solution increases. The effective concentration (EC50) sufficient to scavenge 50% of hydroxyl was calculated and found to be 55.27µg/ml ± 0.67 and 71.36 µg/ml ± 0.87 for ascorbic acid and extract, respectively.

Antimicrobial efficacies of plant extract: The result of antibacterial activity of plant extracts (TE and GE) against *Escherichia coli* and *Bacillus subtilis* with different concentration. The least Zones of inhabitation were displayed by negative control and ciprofloxacin exhibited the wide zone of inhabitation. *Ocimum sanctum* leaves extracts showed increasing zone of inhibition. Results of the study is shown in the **Table 9**.

Table 1: Physico-chemical Characteristics of plant extracts

Physico-chemical Characteristics of plant extracts						
S.No.	Constituents	Chemical Test	Observation		Inference #	
			Ethanolic extracts Ginger rhizomes	Ethanolic extracts Tulsi life	Ginger	Ocimum
01.	Carbohydrates	Molisch's test				
02.	Reducing sugar	Fehiling's test, Benedict's test	No. color changed	No. color changed	–	–
03.	Protein	Biuret test	Green color	No. color changed	+	–
04.	Tannins	Fecl2 test, Lead acetate test, and Potassium dichromate test	Deep blue with Fecl2, White ppt with acetate solution and red ppt with potassium dichromate	Deep blue with Fecl2, White ppt with acetate solution and red ppt with potassium dichromate	–	–
05.	Lipids	Sudan red III	No red color	No red color	–	–
06.	Amino acids	Ninhydrin test	Absent	Purple color	+	–
07.	Alkaloids	Dragndroff's test, Mayer's test, Wagner's test, Hager's test	Orange brown ppt	Orange brown ppt	–	–
			Creamy ppt	Creamy ppt	–	–
			Reddish brown ppt	Reddish brown ppt	–	–
			Yello ppt	Yello ppt	–	–
08.	Saponins	Saponins	Stable	Stable	+	+
09.	Flavonoids	Flavonoids	No change	Yellow ppt	–	+

 # Physiochemical analysis of extracts: "+" Present; "-" Absent.

Table 2: Determination and validation of λ_{max} of various extracts

Extracted part of plant	Solvent used in extraction	Buffer solution used for dilution	Determination and validation of λ_{max} of various extracts																	
			Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
			9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm	9:30 am	12:30 pm	4:30 pm
Tulsi leaves	Chloroform	PBS pH 7.4	227 ±1.89	227 ±189	227.5 ±0.81	278 ±6.64	227.5 ±3.50	404 ±3.54	542.5 ±5.1	540 ±5.50	535.5 ±4.50	445 ±4.84	436.5 ±1.91	356 ±2.89	543 ±3.87	568.5 ±0.00	432 ±1.89	217.5 ±2.50	546 ±1.50	434 ±4.98
		PBS PH 6.8	269.5 ±1.5	227.5 ±2.50	227 ±1.5	230 ±3.5	227.5 ±3.50	228 ±2.45	228 ±1.67	228 ±2.50	228.5 ±1.26	324 ±2.56	227 ±1.05	274 ±3.34	503 ±2.50	273 ±3.90	334.5 ±2.30	228 ±1.89	227.5 ±2.46	453 ±0.47
		0.1N HCL	228 ±0.81	228 ±0.98	288 ±0.79	230 ±1.81	228 ±0.23	228 ±1.50	228 ±0.56	278 ±0.47	234 ±0.67	227.5 ±0.89	227 ±0.67	224 ±0.34	284 ±0.76	235 ±0.87	227.5 ±0.45	335 ±0.98	228 ±0.86	258.5 ±0.78
	n-butanol	PBS pH 7.4	276 ±5.43	483 ±4.49	678 ±9.59	678.5 ±9.74	675.5 ±9.54	676 ±9.53	677.5 ±9.42	679 ±9.53	685 ±9.52	498 ±4.34	480.5 ±4.78	678 ±9.53	786 ±9.99	384 ±3.67	445.5 ±5.73	667 ±9.63	678 ±9.42	545 ±4.53
		PBS PH 6.8	274 ±0.54	271 ±0.81	272.5 ±0.68	272 ±0.52	270.5 ±0.42	205 ±1.56	270 ±2.62	335 ±4.22	243 ±0.81	223 ±0.81	323 ±0.56	227.5 ±2.63	432 ±0.98	287 ±1.56	229 ±1.59	254 ±0.52	287 ±2.73	489 ±2.83
		0.1N HCL	272 ±0.81	276 ±0.45	275 ±0.87	203 ±2.67	274.5 ±1.52	264.5 ±0.78	274.5 ±2.01	268.5 ±2.89	274 ±0.18	226 ±0.81	232 ±2.76	278.5 ±1.62	298 ±0.52	276 ±0.56	309.5 ±0.84	205.5 ±0.67	224 ±2.67	209.5 ±1.78
	Ethanol	PBS pH 7.4	279.5 ±0.47	275 ±0.81	278 ±0.56	276.5 ±1.67	275 ±2.78	671 ±0.81	674.5 ±0.81	654 ±1.52	598.5 ±0.81	509 ±2.89	654 ±1.89	576 ±0.56	487.5 ±0.53	678 ±0.67	452 ±4.90	224 ±3.09	22.5 ±0.56	586 ±1.90
		PBS PH 6.8	279 ±0.47	275 ±0.78	274.5 ±0.81	271.5 ±0.84	272 ±0.67	271 ±2.83	270.5 ±2.09	278.5 ±0.81	289 ±1.78	278.5 ±1.31	298 ±0.78	227 ±1.89	298 ±2.78	270.5 ±2.78	202 ±5.00	278 ±1.62	298.5 ±0.62	298 ±0.52
		0.1N HCL	275.5 ±2.09	279 ±1.24	270.5 ±0.81	203.5 ±1.82	277.5 ±2.78	277 ±0.81	278 ±0.81	298.5 ±1.67	274 ±2.98	287 ±0.18	225.5 ±4.78	289 ±0.53	267 ±2.87	277.5 ±0.65	289.5 ±1.72	278 ±2.89	225 ±3.45	298 ±1.56
Ginger Stem	Ethanol	PBS pH 7.4	270 ±0.81	266 ±1.62	268 ±2.53	271.5 ±2.67	792.5 ±0.76	270 ±1.09	668.5 ±3.32	587 ±0.65	274.5 ±0.23	589 ±0.65	685 ±1.76	456.5 ±3.65	678 ±1.69	658.5 ±0.84	227 ±2.78	267.5 ±1.87	563 ±0.62	730 ±2.83
		PBS PH 6.8	270 ±0.81	272 ±0.81	274 ±1.62	274.5 ±2.73	272 ±0.81	272 ±1.72	272 ±0.81	273 ±3.73	272.5 ±0.81	278 ±1.72	272.5 ±2.02	225 ±0.52	279 ±1.62	275 ±0.62	277.5 ±2.73	276 ±3.93	267 ±0.81	235.5 ±0.81
		0.1N HCL	272.5 ±0.84	273.5 ±0.81	270.5 ±0.62	273 ±0.81	272 ±0.82	269 ±1.84	272.5 ±0.84	273 ±2.63	273.5 ±3.73	278 ±0.81	287 ±0.81	244.5 ±0.84	275 ±1.69	298 ±0.81	278 ±0.84	253.5 ±1.82	243 ±1.89	287 ±1.86

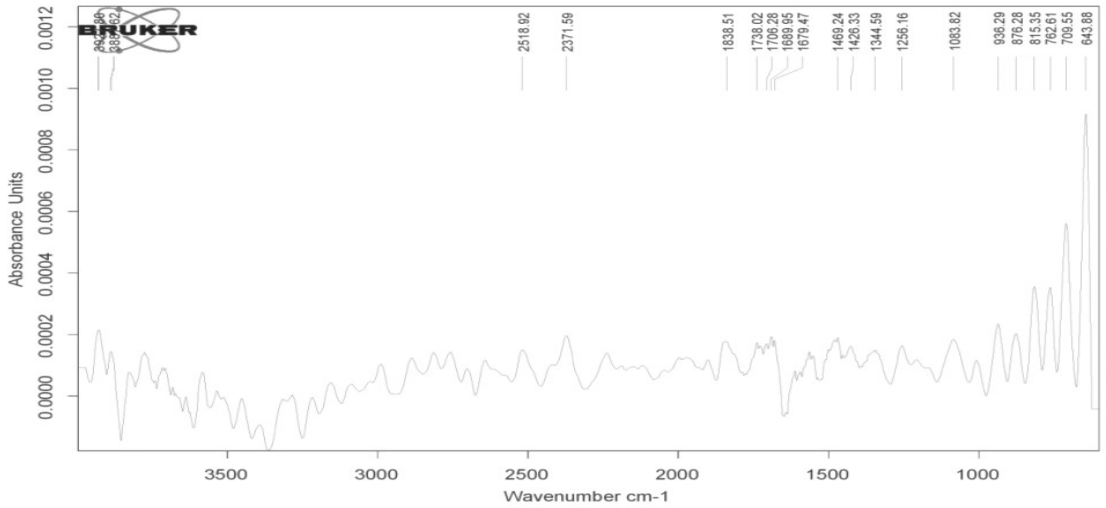

Figure 1: IR Spectra of the Extract of Tulsi chloroform

Table 3: IR Interpretation of the Extract of Tulsi chloroform

S. No.	Wave number (cm^{-1})	Functional groups
1.	3927.80	O-H
2.	2371.59	N-H str of amine and amide
3.	2926.66	C-H For alkane
4.	1426.33	C-H bending
5.	1083.82	Amine C=N str
6.	1679.47	Amino acid N-H str
7.	643.85	Aromatic meta di substituted

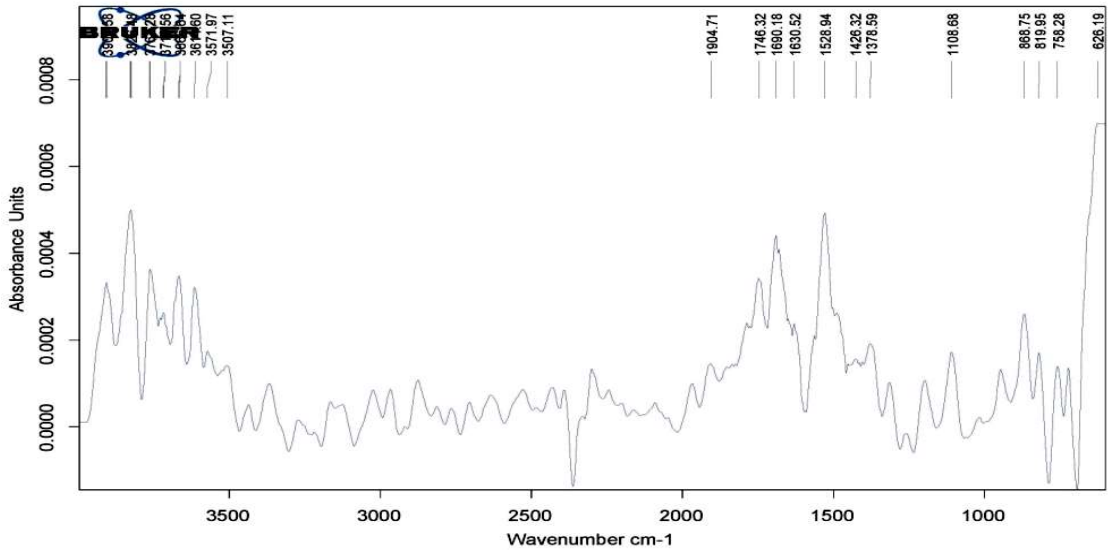

Figure 2: IR Spectra of the Extract of Tulsi n-Hexane

Table 4: IR Interpretation of the Extract of Tulsi n-Hexane

S. No.	Wave number (cm^{-1})	Functional grups
1.	3901.58	O-H
2.	1378.59	Phenol C-O
3.	1746.71	Ester C=O
4.	1426.32	C-H bending
5.	1108.68	Amine C=N str
6.	1690.68	Amino acid N-H str
7.	626.19	Aromatic meta di substituted

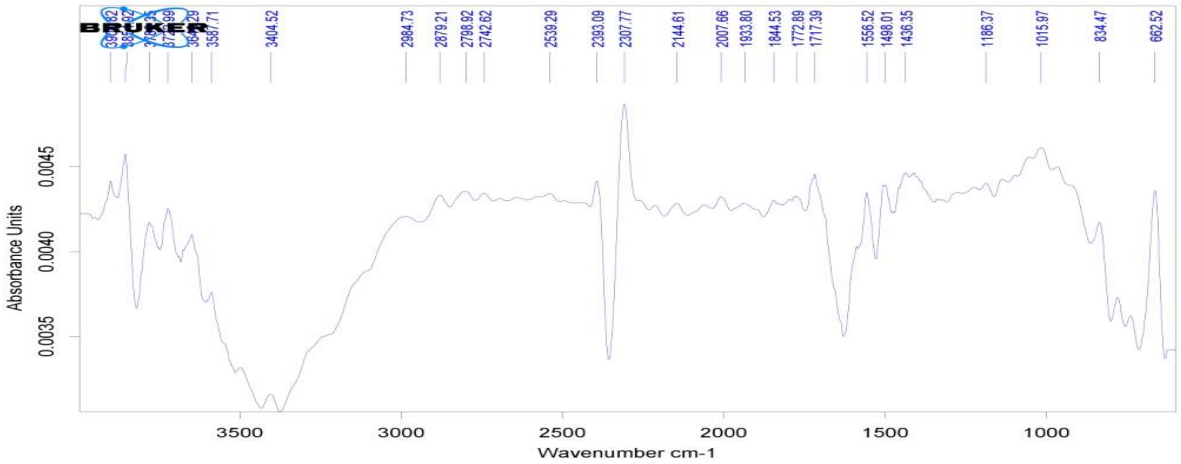

Figure 3: IR Spectra of the Extract of Tulsi n-butanol

Table 5: IR Interpretation of the Extract of Tulsi n-butanol

S.No	Wave number (cm^{-1})	Functional groups
1.	3900.82	O-H
2.	2393.09	N-H str of amine and amide
3.	1844.53	C=O, C=N
4.	1772.89	Ester C=O
5.	1498.01	C-H bending
6.	1772.89	Ester C=O
7.	1498.01	C-H bending
8.	662.52	Aromatic meta di substituted

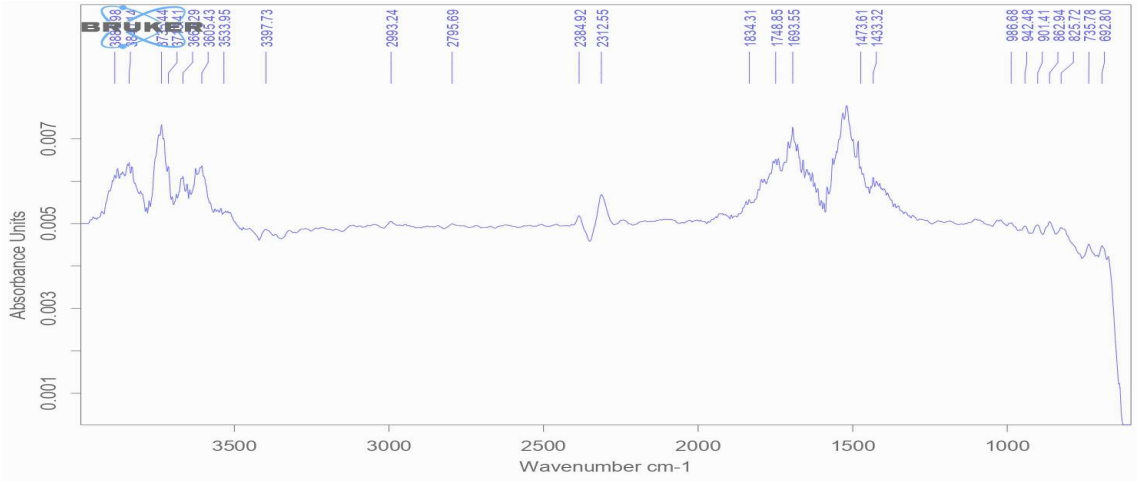

Figure 4: IR Spectra of the Extract of Ginger chloroform

Table 6: IR Interpretation of the Extract of Ginger chloroform

S. No	Wave number (cm^{-1})	Functional groups
1.	3886.98	O-H
2.	1399.36	Phenol C-O
3.	2384.92	N-H str of amine and amide
4.	1834.31	C=O, C=N
5.	1748.85	Ester C=O
6.	1473.61	C-H bending
7.	1693.55	Amino acid N-H str
8.	986.68	C-H
9.	692.80	Aromatic meta di substituted

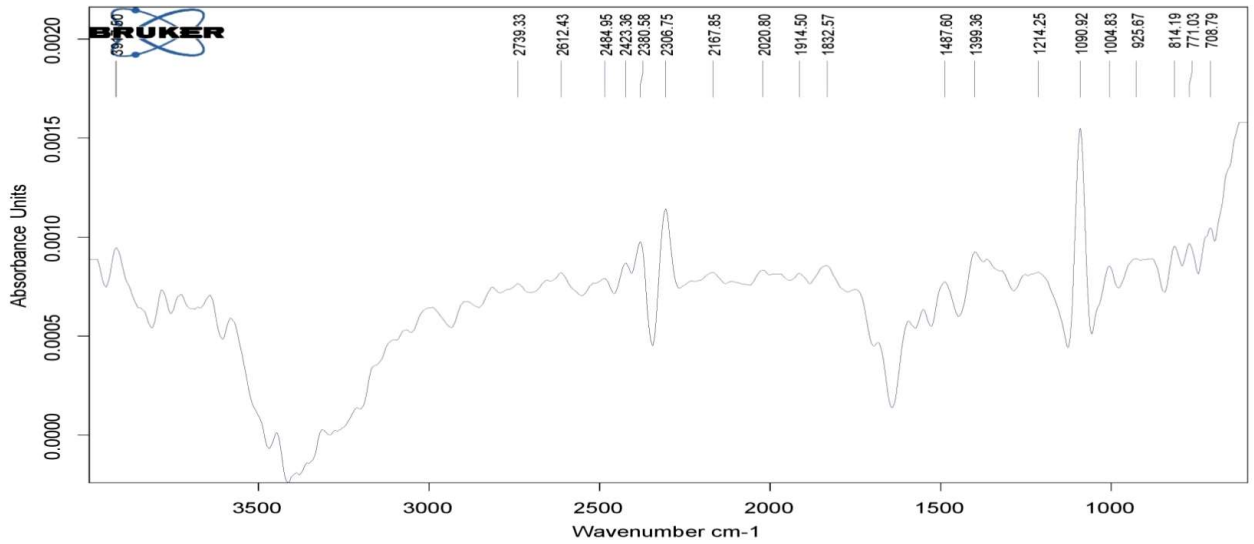

Figure 5: IR Spectra of the Extract of Tulsi ethanol

Table 7: IR Interpretation of the Extract of Tulsi ethanol

S. No.	Wave number (cm^{-1})	Functional groups
1.	3917.50	O-H
2.	1399.36	Phenol C-O
3.	2380.58	N-H str of amine and amide
4.	1214.25	Ether C-O str
5.	1090.92	Ether C=O-OC str
6.	2306.75	C=O str
7.	814.19	Aromatic meta di substituted

Table 8: TLC characterization of Plant extracts

TLC characterization of Plant extract					
Solvent system	Spraying agent/treatment	*Zingiber officinale* Extract		*Ocimum sanctum* Extract	
		No of spots	R_f value	No of spots	R_f value
Choloroform	Iodine chamber and 40% perchloric acid	2	0.77, 0.78	3	0.18, 0.4, 0.58
n-Butanol	Iodine chamber and 40% perchloric acid	0	-	0	-
Benzeen	Iodine chamber and 40% perchloric acid	2	0.37,0.81	2	0.19, 0.82
Choloroform:n-Butanol (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
n-Butanol: benzene (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
Purified Water	Iodine chamber and 40% perchloric acid	0	-	0	-
Water:n-Butanol (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
n-Hexane	Iodine chamber and 40% perchloric acid	0	-	0	-
Benzen:n-Butanol (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
n-Butanol:n-Hexane (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
Benzen:n-Butanol (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
n-Butanol: n-Hexane (1:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
n-Butanol: n-Hexane (2:1)	Iodine chamber and 40% perchloric acid	0	-	0	-
n-Butanol: n-Hexane (4:1)	Iodine chamber and 40% perchloric acid	0	-	0	-

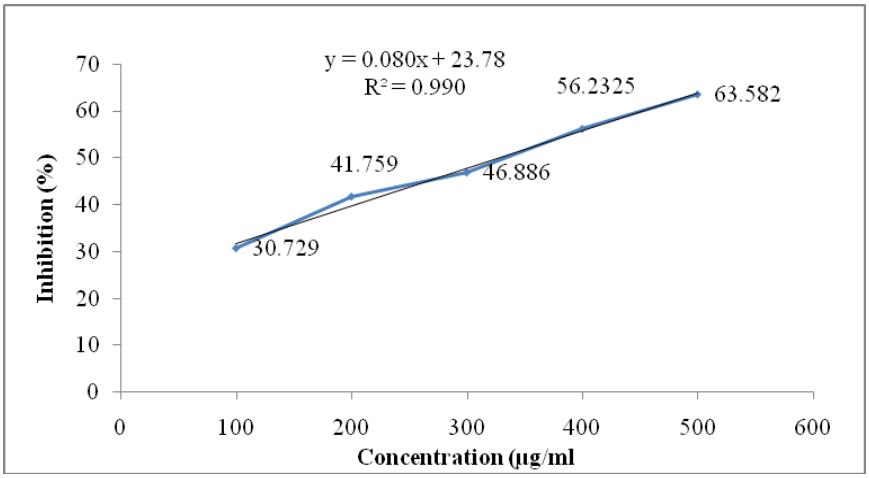

Figure 6: Comparative assessment of antioxidant potential (using DPPH scavenging model) of TE:

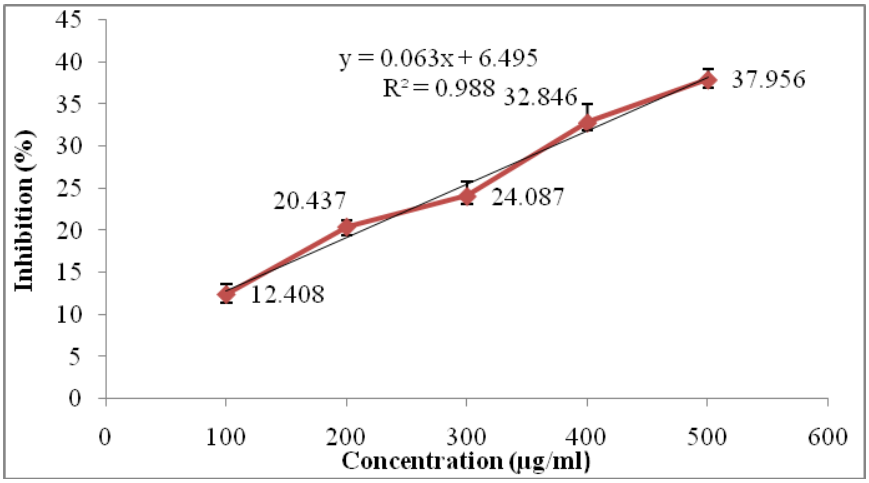

Figure 7: Comparative assessment of antioxidant potential of extract (TE) using H_2O_2 scavenging model

Table 9: Antimicrobial activity of extracts

Bacteria type	Antimicrobial activity of extract											
	Zone of inhibition (mm)											
	Ginger chloroform (µg/ml)		Ginger ethanol (µg/ml)		Tulsi chloroform (µg/ml)		Tulsi ethanol (µg/ml)		Tulsi n-butanol (µg/ml)		Ciprofloxacin (µg/ml)	
	100	200	100	200	100	200	100	200	100	200	100	200
B. Subtilis	2	3	5	9	3	5	4	6	2	2	36	38
E. Coli	2	3	4	4	3	5	4	6	2	2	32	38

CONCLUSION

It can be concluded that *Zingiber officinale* is a good source of antioxidant capered to *Ocimum sanctum linn* and most of the antioxidant components exhibit higher activity in alcoholic media as determined by different assays. It is a low cost extraction method because fewer amounts of solvents used. Phytochemical compound of extracts revealed the presence of alkaloids, tannins, falvonoids, glycosides, saponins, proteins, carbohydrate, terpenoids, and amino acids.

This is responsible for pharmacological activity of extract. Extracts were found to be soluble in 0.1 N HCl, phosphate buffer solution pH 6.8 and phosphate buffer solution pH 7.4, so suitable for maximum possible drug delivery systems. The extracts pH was found to be neutral. So present Study was claimed that extracts were free from heavy metals (lead, arsenic) and microbial load showed high antioxidant activity in both models DPPH scavenging and H_2O_2 scavenging model. So extract can be considered natural source of antioxidant agent. It also be concluded that extracts were having significant antioxidants potential but less than ascorbic acid when used agents DPPH and hydroxyl free radicals.

Conflict of interest: A research of conflict all manuscripts for research reports that are submitted to the journal must be a companied by a conflict of interest disclosure statement.

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