

In Vitro Comparative Study of Antioxidant and Antibacterial Activity of Selected Dietary Plants

Ashok kumar, Palvi Sharma, Anupama Mahajan, Zarina Begum

Department of Biotechnology, *Shaheed Udham Singh College of Engineering and Technology (SUSCET)*, Punjab

Abstract

Ethanol extracts of Garlic (Bulb), Aloe (leaf), Flower bud (buds), Turmeric (rhizomes) and Ginger (rhizomes) were used for relative analysis of antioxidant and antimicrobial activity. Antioxidant activity was determined by DPPH [1, 1-Diphenyl-2-picryl hydrazyl] assay and expressed with Ascorbic acid. It was observed that turmeric and ginger have more antioxidant activity than garlic, Aloe and Flower bud. These extracts were further studied for antibacterial activity by agar well diffusion and spectrophotometric method against tetracycline as reference. The result showed that Flower bud is more effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* compared to other plants extract. However, all the plants extract did show antioxidant and antibacterial activity.

Keywords: Antioxidant, Antimicrobial, Spectrophotometer and Well Diffusion

***Corresponding Author**

Email: ashoka.kumar2007@rediffmail.com

Introduction

The medicinal importance of plants consists of some chemical substances that affect physiological action on human body. Plants play a specific role in traditional medicine which contain phytochemicals that includes alkaloids, flavonoids, terpenoids, steroids, carotenoids and other phenolic compounds [1,2]. These constituents have antioxidant [3] and antimicrobial activity [4] and serve as defence mechanism against many microorganisms [5]. Many of them are used as spices and food supplements [6].

Antioxidants are medicaments, having ability to protect human body from oxidative stress induced by free radicals [7]. It has been found that oxidative stress is major causing factor for initiation of many diseases including neuro degenerative, diabetes, cancers and others [8, 9]. The oxidation of lipid, protein and carbohydrates by toxic reactive species, cause DNA mutation and lead to damage the cell/tissue and death [10]. Free radicals, stimulate oxidative stress and can obviate with antioxidants. Antioxidants will be effective in scavenging of free radicals and suppress such disorder [11]. It is found in most of the plants and due to its natural source, have expected to gain comparison with synthetic antioxidants [12]. The

natural antioxidants do not induce side effect while synthetic antioxidant induce genotoxicity [13].

These plants are also useful as curative agent against numerous pathogenic infections because of its phytochemicals or secondary metabolites [14, 15]. A number of phytochemicals like anthocyanins, flavonoids and other phenolic compounds have antimicrobial activity. Due to drug resistance of pathogens, efforts have been made to find their substitute for treatment of diseases and with knowledge of antimicrobial activity of plant extracts, they may play a significant role in cure of microbial diseases [16]. Presently, herbs are used as natural antimicrobial and antioxidant resources [17]. In the present study, five plants viz. Garlic (Bulb), Aloe (leaf), Flower bud (buds), Turmeric (rhizoids), Ginger (rhizoids) are studied which are being used as supplement and herbal medicine world-wide.

The aim of present research was to investigate phytochemicals in extracts of different plant as well as to examine antioxidant and antimicrobial activity using in vitro system. There is also comparison of well diffusion method with spectrophotometric method for antimicrobial activity.

Table 1: A brief summary of plants.

Plant	Family	Medicinal use	Reference
Garlic (<i>Alium sativum</i>)	Amaryllidaceae	It has antiseptic, anticoagulant, antibiotic, antioxidant, analgesic, anti-inflammatory and anti-cancerous properties.	[18, 19, 20]
Ginger (<i>Zingiber officinale</i>)	Zingiberaceae	It has antimicrobial, antioxidant, anti-cancerous, anti-inflammatory.	[21,22,23,24]
Turmeric (<i>Curcuma longa</i>)	Zingiberaceae	Its expressed antimicrobial, antioxidant, anti-cancerous, anti-inflammatory, anti-diabetic.	[25,26,27]
Clove -Flower bud (<i>Syzygium aromaticum</i>)	Myrtaceae	It possesses antimicrobial, antioxidant and anti-inflammatory activities.	[28,29,30]
Aloe vera (<i>Aloe barbadensis miller</i>)	Asphodelaceae	It has antiseptic, antibiotic, antioxidant, analgesic, anti-inflammatory and anti-cancerous properties.	[31,32,33]

Table 2: Protocol for phytochemical screening

Component exit test	Protocol for test	Result for confirmation
Alkaloids	1.0ml extract + 3.0 drops of Dragendoff's reagent	Orange-red precipitate
Flavonoids	1.0ml extract + few drops of dil. NaOH	Intense yellow colour
Total phenol	1.0ml extract + 2.0ml water + few drops of 10% FeCl ₃	Blue-green colour
Tannins	100mg solvent free extract + 1ml 5% FeCl ₃	Bluish- black precipitate
Saponins	1.0ml extract + 20ml water + agitation for 15min.	1cm layer of foam
Steroids	1.0ml extract + 10ml CHCl ₃ + 10ml conc. H ₂ SO ₄	Upper layer - red and lower layer - yellow-green
Terpenoids	5.0ml extract + 2.0ml CHCl ₃ + 3.0ml conc. H ₂ SO ₄	Reddish brown precipitate
Carbohydrate	2.0ml extract + 2 drops of Molish reagent (95% α-naphthol in ethanol) + 2.0ml conc. H ₂ SO ₄	Purple colour at junction
Glycosides	5.0ml extract + 2.0ml CHCl ₃ + 2.0ml CH ₃ COOH	Violet, blue to green colour

Material and Methods

Collection of Herbal Plants

Wild species of Garlic, Ginger, Turmeric, Flower bud and Aloe were taken fresh from the fields. Four bacterial strains such as *Escherichia coli* (MTCC 25922), *Bacillus subtilis* (MTCC 3256), *Pseudomonas aeruginosa* (MTCC 1688) and *Staphylococcus aureus* (MTCC 6810) were used in study.

Preparation of extract

The herbal plants including Garlic, Ginger, Turmeric, Flower bud and Aloe were washed with distilled water and allow drying in the absence of sunlight at 30 -35°C. Dried samples were crushed in powdered form for extraction. Samples were put in soxhlet apparatus with 90% ethanol and extraction was completed. The extracts were dried under vacuum. The thick paste crude drugs were considered as 100% concentration of extract. Each extract were dissolved in 10% DMSO (Dimethylsulfoxide) for further use.

Phytochemical Screening of Plants-

Phytochemical investigation of extracts (10% DMSO) were performed as per described earlier [34,35].

Antioxidant Activity

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH-0.002%) method [36, 37]. The diluted working solution of the test extracts (10 mg/ml) was prepared using the respective solvents. 10mg/ml of Ascorbic acid used as reference for compare results of plant extracts. In 3ml of total reaction solution, 2ml of extract/standard solution and 1.0ml of DPPH were mixed and allowed to react at 37°C for 30 min. Afterward, absorbance value were measured at 520nm and converted into percent antioxidant activity. The percentage antioxidant activity was calculated by following formula.

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = (A - B)/A * 100$$

Where A = Absorbance of the blank and B = Absorbance of the sample

Table 3: Phytochemical screening of plants extract

Phytochemicals	Herbal Plants				
	Turmeric	Garlic	Ginger	Aloe	Flower bud
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	-
Saponins	-	+	+	+	+
Steroids	+	+	-	+	-
Tanins	-	-	+	+	+
Glycosides	-	+	+	+	+
Terpenoids	+	+	+	+	+
Alkaloids	-	+	+	-	+
Carbohydrates	-	+	+	+	+

Antibacterial Activity

Four pathogenic bacteria namely *Escherichia coli* (MTCC 25922), *Bacillus subtilis* (MTCC 3256), *Pseudomonas aeruginosa* and *Staphylococcus aureus* (MTCC 6810) were collected from MTCC, Chandigarh. The obtained cultures were further sub-cultured for test. For preparation of inoculums, they were sub-cultured in test tube containing 10ml nutrient broth and standardized with saline water.

Agar well diffusion method

Agar well diffusion [38] was used with manipulation. 0.5ml (9.0×10^4 CFU/ml) of 48 hrs old cultures of test organisms were inoculated into different sterile petri-plates and near about 20 ml sterile media was poured into each dish. The dishes were gently shaken for proper mixing and allowed to solidify. Thereafter, four wells were punched of 5 mm diameter with a sterilized cork borer. For each well 50 μ l of different extracts were added. The plates were incubated at 37 °C for 24 hrs and then zone of inhibition in mm was measured. The experiment was carried out in triplicates.

Spectrophotometric method

0.5ml (9.0×10^4 CFU/ml) strains of 48 hrs old cultures were inoculated in test tubes with 20 ml culture media. To each tube, 50 μ l of different extracts were added and incubated for 24 hrs. Optical density of grown bacteria was measured at 450 nm wavelength. Tetracycline (50 μ g/ml) was included as control in this protocol [39, 40].

Result

The comparative study of antimicrobial and antioxidant activity was conducted with dietary

plants like Garlic, Ginger, Turmeric, Flower bud and Aloe. We have also evaluated the antimicrobial activity by Disc diffusion and spectrophotometric method. All the experiment were done in triplicates.

Phytochemical

The phytochemical analysis is preliminary characterization of plant extracts which have bioactive compounds. The phytochemical screening show that all the experimental plants contain phenols and terpenoids. Turmeric extract does not contain Saponins, tannins, glycosides, alkaloids and carbohydrate. The Garlic extract contain all the phytochemicals except tannins as listed below whereas Ginger extract don't have steroids and Flower bud extract contain all phytochemical except flavonoids and steroids. Aloe contains all the phytochemical such as phenols, flavonoids, Saponins, tannins, glycosides, terpenoids, alkaloids and carbohydrates.

Antioxidant Activity

Among the five extract of plants, Ginger showed maximum antioxidant activity and Aloe showed the lowest activity and reported as 51.54% for Turmeric, 28.17% for Garlic, 54.08% for Ginger, 10.97% for Aloe, 19.41% for Flower bud. Ascorbic acid standard showed 80.5% activity. Further experiments are needed to confirm which phytochemicals show antioxidant activity. There are major articles available that report that these plants extract have antioxidant activity [13, 15, 26, 33].

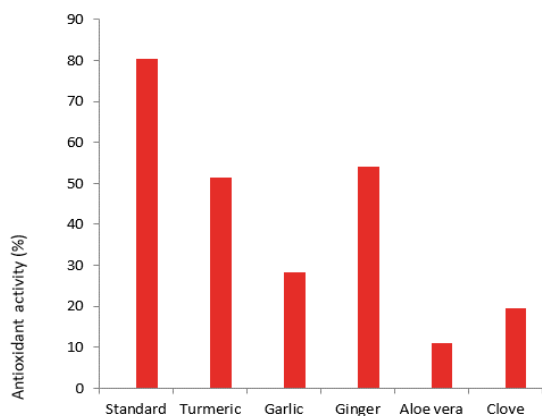


Figure 1: Antioxidant Activity of plants extract (10mg/ml) with Ascorbic Acid (10mg/ml) as standard

Antimicrobial Activity

Agar well diffusion method

Table 4 shows the antimicrobial activity of selected plant extracts by agar cup diffusion method and the result is similar to that of spectrophotometric method. The result reveal that Garlic extract inhibit maximum growth of *E. coli* whereas *Pseudomonas aeruginosa* is maximum inhibited by Aloe and Flower bud extract inhibit maximum growth of *Bacillus subtilis* whereas *Staphylococcus aureus* is maximum inhibited by Ginger.

Spectrophotometric method

The antimicrobial activity of ethanolic extract of Turmeric, Garlic, Ginger, Aloe and Flower bud were tested against bacterial strain such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus*

B. subtilis and *Staphylococcus auerus* by spectrophotometer. Tetracycline was used as standard. *Escherichia coli* showed maximum inhibition against Garlic extract whereas minimum inhibition was against Aloe. *Pseudomonas aeuroginosa* have maximum inhibition against Aloe whereas minimum inhibition was against Turmeric extract. *Bacillus subtilis* showed maximum inhibition against Flower bud extract whereas Turmeric showed minimum inhibition. *Staphylococcus auerus* showed maximum inhibition against Ginger whereas Turmeric extract showed minimum inhibition.

Discussion

The phytochemical screening result revealed that ethanolic extracts of plants consist phenols, flavonoids, Saponins, tannins, glycosides, terpenoids, alkaloids and carbohydrates and this was confirmed by chemical.

With antimicrobial result [41], it was observed that all of ethanol extracts of plants demonstrated good antibacterial activity. The result of antimicrobial activity expressed significant result against bacterial strains. From determination of antimicrobial activity it was observed that growth of *E. coli* and *S. auerus* is inhibited most by Garlic whereas *P. aeuroginosa* with Aloe and Flower bud inhibit growth of *B. Subtilis* the most. Turmeric and Ginger also showed significant antimicrobial activity. In comparing the well diffusion and spectrophotometric method, well

Table 4: Antimicrobial Activity (ZOI in mm) of plants extract by agar well diffusion method

Micro-organism	Standard (Tetracycline - 50µg/ml)	Turmeric- 50mg/ml	Garlic- 50mg/ml	Ginger- 50mg/ml	Aloe- 50mg/ml	Flower bud- 50mg/ml
<i>E.coli</i>	20.00	9.00	11.00	10.00	9.00	10.00
<i>P. aeuroginosa</i>	22.00	8.00	9.00	8.00	13.00	9.00
<i>B. subtilis</i>	19.00	9.00	13.00	9.00	11.00	15.00
<i>S. aureus</i>	20.00	10.00	13.00	14.00	12.00	13.00

Table 5: Antimicrobial Activity (ZOI in Optical density) of plants extract by spectrophotometric method

Micro-organism	Standard (Tetracycline- 50µg/ml)	Turmeric- 50mg/ml	Garlic 50mg/ml	Ginger- 50mg/ml	Aloe- 50mg/ml	Flower bud- 50mg/ml	Positive
<i>E.coli</i> (-)	0.093	0.309	0.272	0.344	0.499	0.378	0.842
<i>P. aeruginosa</i>	0.110	0.580	0.430	0.361	0.303	0.521	0.806
<i>B. subtilis</i>	0.092	0.460	0.257	0.236	0.362	0.066	0.741
<i>S. aureus</i>	0.042	0.360	0.197	0.136	0.162	0.259	0.714

diffusion method has ability to evaluate the activity of antimicrobial drugs including plant extracts. Although, this method has no significant difference from other methods in some report. Spectrophotometric method [39], of finding the inhibitory action of drugs and extracts is fast and comfortable to use. Statistically, there is no significant difference between result obtained by both method. So, spectrophotometric method can be recommend as suitable and sensitive method for investigation of antimicrobial activity of extracts.

The comparative investigation was conducted between selected plants Turmeric, Garlic, Ginger, Aloe and Flower bud for antioxidant activity. The scavenging of DPPH radical is most used protocol to evaluate free radical scavenging ability of plant extracts [42]. From obtained result, it was seen that Ginger (54.08%) and Turmeric (51.54%) expressed better antioxidant effect although all are good antioxidants. Thus, tendency of antioxidant was Ascorbic acid > Ginger > Turmeric > Garlic > Flower bud > Aloe in decreasing order. This observation proved that all the selected plants are free radical inhibitors and possibly act as primary antioxidant in human being. The antioxidant effect on DPPH is due to the ability of their hydrogen donation and it has been observed that plant extracts expressed proton donating ability and serve as free radical scavengers and work as possible primary antioxidant. Although, percent of antioxidant ability of extracts were not greater than standard.

The obtained result might be sufficient for further identification of active phytochemicals and evaluate their antimicrobial and antioxidant activity. The report of present study revealed that all pathogenic microbes have different sensitivity against individual extract, added in growth medium.

Conclusion

In conclusion, the work revealed that ethanolic extracts of plants are dissimilar in their antimicrobial and antioxidant properties. The study revealed that Turmeric and Ginger have higher antioxidant activity but there is variation in antimicrobial activity with specific organism.

However, further studies are necessary *in vitro* and *in vivo* to support this suggestion. Further, Phytochemicals analysis should be done to qualify and quantify the component which possess antimicrobial and antioxidant activity at molecular level and their possible mechanism of action. This result show that these plants should be good source of antimicrobial and antioxidant agents and spectrophotometric technique is effective for determination of antibacterial activity of extracts.

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