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STUDIES ON *IN VITRO* ANTIOXIDANT AND ANTISTAPHYLOCOCCAL ACTIVITIES OF SOME IMPORTANT MEDICINAL PLANTS

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Abstract

Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules in healthy human cells and cause protein and DNA damage along with lipid peroxidation. Many phytochemicals have been found to play as potential antioxidants and antimicrobials**.** In the present study antioxidant and antistaphylococcal activities of *Bauhinia variegata*, *Tinospora cardifolia* and *Piper longum* have been determined. Total phenolic contents in plant extracts were estimated and different amounts of phenolic contents were found in *B. variegata*, *T. cardifolia* and *P. longum* extracts. The antioxidant activity of the extracts was compared with standard antioxidants such as, BHA, BHT, quercetin, ascorbic acid and propyl gallate. The % scavenging activity gradually increased with increasing concentrations of the test extracts in DPPH radical scavenging assay. Dose dependent antioxidant activity pattern was also observed in phosphomolybdate assay. Antioxidant activity was directly correlated with the amount of total phenolic contents in the extracts. As compared to *B. variegata*, the extracts from other two plants exhibited higher antioxidant activity. In disc diffusion assays several solvent extracts derived from test plants inhibited the growth of *Staphylococcus aureus*. Maximum inhibitory efficacy was observed in *T. cardifolia* extracts. However, the lowest minimum bactericidal concentration (MBC) (0.43 mg/ml) was recorded for ethyl acetate and acetone extracts of *P. longum*. This study demonstrates notable antioxidant and anti-staphylococcal roles assigned to some plant extracts tested.

Key words: Free radicals, antioxidant, antibacterial, *Bauhinia variegata*, *Tinospora cardifolia*, *Piper longum*, *Staphylococcus aureus*.

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Abbreviations: **RSA** : radical scavenging assay ; **ROS** : reactive oxygen species ; **PE** : petroleum ether ; **BZ** : benzene ; **CH** : chloroform ; **EA** : ethyl acetate ; **AC** : acetone ; **ET** : ethyl alcohol ; **AQ** : water ; **PGE** : propyl gallate equivalent ; **BHT** : butylated hydroxytoluene ; **BHA** : butylated hydroxyanisole ; **MHA** : Mueller Hinton agar ; **MBC** : minimum bactericidal concentration ; **AO** : antioxidant ; **ZOI** : zone of inhibition.

INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (47). The most common reactive oxygen species (ROS) include superoxide (0_2^-) anion, hydrogen peroxide (H_20_2), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO^-) and peroxynitrite anion ($ONOO^-$). Majority of the diseases/disorders are mainly linked to oxidative stress produced due to free radicals (15). ROS have been implicated in over a hundreds of disease states which range from arthritis, connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (21). Antioxidant

therapy has gained an immense importance in the treatment of these diseases. Antioxidants have been reported to prevent oxidative damage caused by free radicals and ROS, and may prevent the occurrence of diseases such as cancer and aging. They can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also acting as oxygen scavengers (8, 41). Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase, and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and polyphenols which are obtained from natural plant sources (24). A wide range of antioxidants from both natural and synthetic origin have been proposed for use in the treatment of various human diseases (9). Some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone are commonly used in processed foods. However, these compounds have been shown to produce toxic effects like liver damage and mutagenesis (14, 49). Hence, nowadays search for natural antioxidant source is gaining much importance.

Infectious diseases are still a major threat to public health, despite the tremendous progress made in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance (31). Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to treat many infectious diseases (18). Therefore interest in higher plant extracts exhibiting antimicrobial activity has increased in recent years (4, 5, 12)**.** Staphylococci, especially *Staphylococcus aureus*, are among the most common causes of nosocomial as well as community-acquired infections. *S. aureus,* a common coloniser of human skin and mucosa, can cause diseases such as skin and wound infections, urinary tract infections, pneumonia and bacteraemia (blood stream infection). It can also cause food poisoning. Most strains of this bacterium are sensitive to many antibiotics, but some *S. aureus* bacteria are resistant to the antibiotic methicillin. Many phytochemicals have been reported to possess antibacterial activities against *S. aureus* (32, 33).

Tinospora cordifolia (family Menispermaceae) is used in Ayurvedic and Jamu herbal medicine as a hepatoprotectant. The extracts of *T. cordifolia* and turmeric have been shown to prevent the hepatotoxicity resulting from conventional pharmaceutical treatments of tuberculosis using drugs such as isoniazid and rifampicin (1). Spices have also been reported to produce variety of medicinal effects (27). The dried fruits of *Piper longum* (long pepper, family Piperaceae) are used as a spice and seasoning material. *P. longum* is a component of medicines reported as good remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain, and arthritic conditions (45). Other reported beneficial effects of *P. longum* include analgesic and diuretic effects, relaxation of muscle tension, and alleviation of anxiety. Bark of *Bauhinia variegata* Linn. (family Leguminosae) is traditionally used as tonic and in treatment of ulcers. It is also useful in skin diseases. The roots are used as antidote to snake poison (19). In folklore medicine, this plant is also used for managing inflammatory conditions (37). Present study was, therefore, carried out to evaluate the antioxidant and antistaphylococcal activities of the extracts derived from medicinal plants namely *B. variegata*, *T. cordifolia*, and *P. longum* using various *in vitro* models. Phenolic content in extracts was also determined.

MATERIALS AND METHODS

Chemicals

L-ascorbic acid, propylgallate, BHA (butylated hydroxyl anisole), tert-butyl-4-hydroxy toluene (BHT), 1, 1-Diphenyl-2-picryl hydrazyl (DPPH), quercetin, nutrient agar and Mueller-Hinton agar were purchased from Himedia (Mumbai, India). Methanol, DMSO and other organic solvents were purchased from Merck (India).

Plant materials

Leaves of the *B. variegata* and stem of the *T. cordifolia* were collected from botanical garden of the University of Allahabad, Allahabad. The fruit part of the plant *P. longum* was purchased from local market. The plant materials were shade-dried at room temperature for 10-15 days. Dried leaves, stems and fruits were separately crushed and ground into fine powder with mortar and pestle.

Extraction

Fifty grams of each powdered sample was extracted with petroleum ether (PE), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC), ethyl alcohol (ET) and

water (AQ) in Soxhlet apparatus for 6 h according to the method described elsewhere (28, 32). The respective extracts were centrifuged and filtered. Solvent was removed completely under reduced pressure. The dried residues were dissolved in DMSO for determination of antioxidant and antibacterial activities of extracts.

Determination of total phenolics

The amount of total phenol in extracts was determined according to the protocols (40, 44) with certain modifications. Two milligrams of each solvent extracts was dissolved in 1 ml DMSO separately for phenol estimation. Samples (0.2 ml) were diluted to 3 ml with water. Small amount (0.5 ml) of two-fold-diluted Folin-Ciocalteu reagent was added and the contents were mixed. After 3 min, 2 ml of 20% Na_2CO_3 solution was added and the tubes were placed in boiling water bath for one min followed by cooling. The absorbance was measured at 650 nm against a reagent blank using UV-visible spectrophotometer (Elico-159). Standard curve was prepared using different concentrations of propyl gallate. The concentration of phenols in the test samples was expressed as mg propyl gallate equivalents/g material (mg PGE/g). The estimation of phenolics in the extract fractions was carried out in triplicate, and the results were expressed as mean ± SEM.

Determination of antioxidant activity in vitro
DPPH radical scavenging assay

The free radical scavenging activity of the extract fractions was measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (44) as modified by us. Three milliliter of 0.1 mM DPPH solution prepared in methanol was added to 0.5 ml of the test extracts (500-2000µg/ml) dissolved in DMSO. The content was mixed and allowed to stand at room temperature for 30 min in dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. The % scavenging activity at different concentrations was determined. BHA, BHT, quercetin and ascorbic acid were used as standard antioxidants for comparison. The percentage scavenging activities (% inhibition) at three different concentrations of the extract fractions were calculated using the following formula, % I = [(Ac – As) / Ac] x 100 where I is inhibition, Ac and As are the absorbance values of the control and the sample, respectively. Three replicates were made for each sample and results were expressed as mean ± SEM.

Antioxidant capacity determination by Phosphomolybdate method

The total antioxidant capacity of the extract fractions was determined by phosphomolybdate method using propyl gallate as standard (30) with modification. To an aliquot of 0.05 ml (20-100 µg) of the extract solution prepared in DMSO, 0.25 ml methanol was added followed by the addition of 3 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95^{o}C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as µg propyl gallate equivalents per gram of sample (µg PGE/g of sample) by using the standard graph. The results were expressed as mean ± SEM (n = 3).

Microbiological assay
Bacterial Culture

S. aureus strain was obtained from the Clinical Microbiology Laboratory, MLN Medical College, Allahabad, India. The bacterial culture was maintained at 4^0C on nutrient agar slants.

Determination of growth inhibitory activity of extracts

The disc diffusion method (27) was used to screen the activity of plant extracts against Gram positive *S. aureus*. The inoculum suspension of bacterial strain was swabbed on the entire surface of Mueller-Hinton agar (MHA). Sterile 6-mm diameter paper discs (Himedia) saturated with 20 µl of extracts prepared in DMSO (containing 3.33 to 10 mg extract/disc) were aseptically placed on the upper layer of the inoculated MHA surfaces. Vancomycin (30 µg) discs (Himedia) were used as standard antibacterial agents for comparison. Discs containing sterile DMSO were used as negative control. Plates were incubated at 37^0C for 18 h and antistaphylococcal activity was determined by measuring diameter of the zone of inhibition (ZOI) surrounding discs. The tests were performed in triplicate and the results were averaged.

Determination of the minimum bactericidal concentration (MBC)

The minimal bactericidal concentration (MBC) of extract fractions was determined using the broth micro-dilution techniques (34). Nutrient broth containing extracts (prepared in DMSO) were two-fold diluted successively and then 100 µl bacterial suspensions having 10^6 CFU/ml (values of 0.08–0.10 at 625 nm, according to McFarland turbidity standards) were added in each tube. The concentration of samples in tubes varied from 121.2 mg/ml to 0.43 mg/ml. The tubes were incubated at 37^0C for 24 h. The same tests were performed simultaneously for growth control (nutrient broth + bacteria). For MBC determination 100µl inoculum suspension taken from different dilution tubes was subcultured on blood agar (for Gram positive *Staphylococci*) and plates were incubated at 37^0C for 24 h. The highest dilution (minimum amount of extract) showing complete absence of growth was recorded as MBC.

RESULTS

Table 1 shows the phenolic contents present in all the extract samples. In *B. variegata*, the phenolic content of the ethanol (ET) extract was maximum (145 mg PGE/g of sample) followed by acetone (AC) and water (AQ) extracts. The phenolic contents were low in most of the non-polar fractions such as petroleum ether (PE), chloroform (CH) and benzene (BZ) extracts. High concentration of phenolics in *T. cordifolia* were obtained in ethyl acetate (EA), acetone (AC), CH and BZ extracts. The values ranged between 17.50 – 92.50 mg PGE/g. The yield of phenolic contents in *P. longum* was high in AQ (135 mg PGE/g) and ET (102 mg PGE/g) extracts.

Table 1. Contents of total phenol in plant extracts

Extract fractions	*B. variegata* (leaf)	*T. cardifolia* (stem)	*P. longum* (fruit)
PE	22.50 ±0.14	25.00 ±0.28	57.50 ±0.13
BZ	65.00 ±0.25	77.50 ±0.05	72.50 ±0.12
CH	45.00 ±0.19	77.50 ±0.05	42.50 ±0.17
EA	57.50 ±0.16	92.50 ±0.02	17.50 ±0.07
AC	127.50 ±0.12	82.50 ±0.13	22.50 ±0.01
ET	145.00 ±0.03	45.00 ±0.02	102.50 ±0.01
AQ	95.00±0.03	17.50 ±0.11	135.00±0.29

The values are represented as mg propyl gallate equivalent per gram of sample (mg PGE/g). The results are expressed as mean ± SEM (n = 3). Abbreviations: PE - petroleum ether, BZ - benzene, CH - chloroform, EA - ethyl acetate, AC - acetone, ET - ethyl alcohol and AQ - water.

Free radical scavenging potentials of *B. variegata*, *T. cordifolia* and *P. longum* extracts at different concentrations (500, 1000 and 2000 µg/ml) were tested by the DPPH method, and the results are shown in Figures 1, 2 and 3, respectively. The degree of discoloration indicates the scavenging potentials of the extracts. At 500µg/ml concentration ET, AC and EA extracts of *B. variegata* (Fig. 1) exhibited about 86, 77 and 24% free radical scavenging activities, respectively. At 1000 µg/ml, ET and AC extracts exhibited about 88-89% free radical scavenging activity while PE and AQ extracts showed minimal activity. Further increase in activity was observed at 2000 µg/ml concentration for all the leaf extracts. In general, with increasing concentration of *B. variegata* extracts, there was gradual increase in free radical scavenging activity. The order of activity of extracts may be represented as ET>AC>BZ≥EA≥CH>PE>AQ.

T. cardifolia extracts also exhibited potential antioxidant response by scavenging free radicals (Fig. 2). Most of the non-polar extract fractions produced good activities. EA, CH, and BZ extracts of the stem demonstrated about 50 – 90% free radical scavenging activity. The scavenging response increased with increasing concentration of extracts. The lowest activity was observed in PE fraction. The order of antioxidant activity in *T. cardifolia* was recorded as EA>CH>BZ>AC>ET>AQ>PE. At highest test concentration (2000 µg/ml) ET and AC extracts produced 54% and 85% free radical scavenging activities, respectively (Fig. 2).

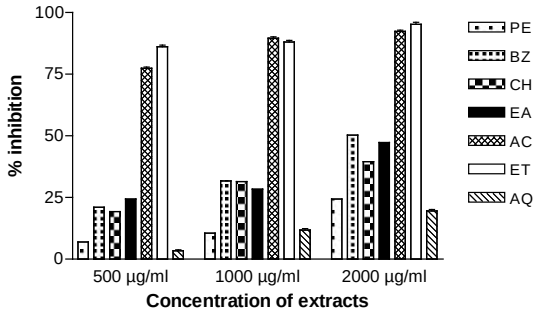

Figure 1. Free radical scavenging activity of *B. variegata* extracts (Antioxidant activity was measured by DPPH radical scavenging assay. Various components present in leaf samples were extracted with petroleum ether (PE), benzene (BZ), chloroform (CH), ethyl acetate (EA), acetone (AC), ethyl alcohol (ET) and water (AQ) as described in methods section. The results are expressed as mean ± SEM of three replicates).

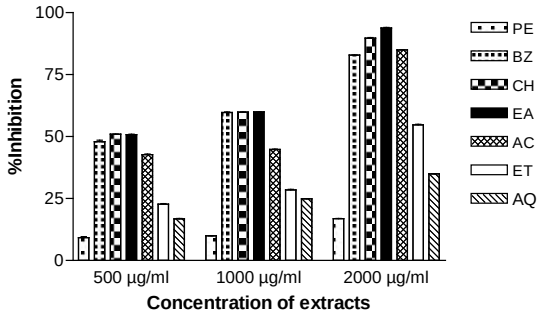

Figure 2. Free radical scavenging activity of *T. cardifolia* extracts (Antioxidant activity was measured by DPPH radical scavenging assay. Various components present in stem samples were extracted with PE, BZ, CH, EA, AC, ET and AQ as described in methods section. The results are expressed as mean ± SEM of three replicates).

P. longum extracts acted like the best free radical scavengers in *in vitro* assays (Fig. 3). At 500 µg/ml concentration, the lowest concentration of the extracts tested, most of the test extracts produced free radical scavenging response in the range of 50-85% except PE extracts (45.42% activity). In general, the best response was observed with AQ extracts. The *in vitro* antioxidant activity of fruit extracts exhibited dose-dependent response. However a very little increment in activity of PE and EA extracts was observed at higher concentrations. At 1000 and 2000 µg/ml concentrations, the scavenging activities of other *P. longum* extracts ranged between 58.5-92% and 76-96%, respectively. The order of free radical scavenging activity was found to be AQ>ET>CH>AC≈BZ>EA>PE. In comparison with different extracts of medicinal plants, standard antioxidant compounds namely, BHA, BHT, quercetin and ascorbic acid exhibited about 95-99% scavenging activities even at 500 µg/ml concentration.

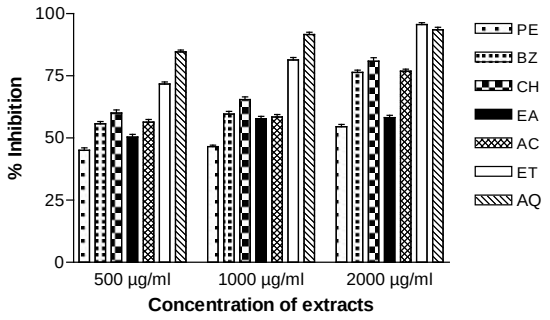

Figure 3. Free radical scavenging activity of *P. longum* extracts (Antioxidant activity was measured by DPPH radical scavenging assay. Various components present in fruit samples were extracted with PE, BZ, CH, EA, AC, ET and AQ as described in methods section. The results are expressed as mean ± SEM of three replicates).

The extracts derived from *B. variegata* leaf samples exhibited various degrees of antioxidant (AO) capacity (Fig. 4). The AO capacity of ET, AC and CH extracts was 257.5, 163.6 and 251.7 µg PGE/g of extract respectively, at 333 ppm. Lowest activity (5.7-55.5 µg PGE/g) was observed in AQ extracts at all four test concentrations. *T. cordifolia* stem extracts possessed better AO capacity in comparison with *B. variegata* extracts (Fig. 5). The AC, EA and CH extracts produced almost similar AO capacity values at all test concentrations. At highest concentrations AC, ET, EA and BZ extracts of *T. cardifolia* exhibited better AO capacity values between 400-500 mg PGE/g of samples. Lowest activity was recorded in AQ and PE extracts.

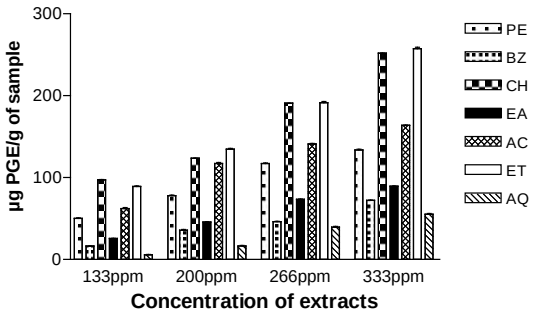

Figure 4. Antioxidant (AO) capacity determination of *B. variegata* extracts by Phosphomolybdate method (Various components present in leaf samples were extracted with PE, BZ, CH, EA, AC, ET and AQ as described in methods section. The AO capacity was expressed as µg PGE/g of sample. Each value is represented as mean ± SEM of three replicates).

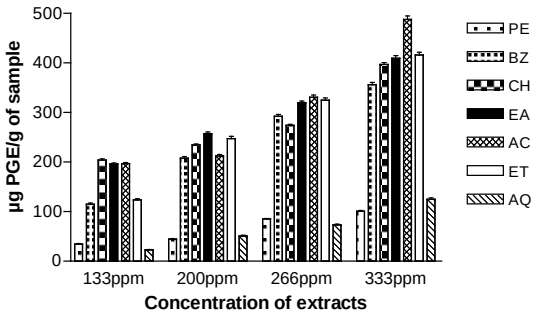

Figure 5. Antioxidant capacity determination of *T. cardifolia* extracts by Phosphomolybdate method (Various components present in stem samples were extracted with PE, BZ, CH, EA, AC, ET and AQ as described in methods section. The AO capacity was expressed as µg PGE/g of sample. Each value is represented as mean ± SEM of three replicates).

P. longum extracts exhibited very high antioxidant capacity *in vitro* (Fig. 6). Therefore AO capacity of *P. longum* extracts was tested at lower concentrations of test extracts (26.66 ppm, 40 ppm, 53.33 ppm and 66.66 ppm). ET, CH and PE extracts produced appreciable responses even at lower extract concentrations. BZ, AC and AQ fractions exhibited considerably better responses at higher test concentrations (66.66 ppm) producing 604.4, 420.5 and 408.6 mg PGE/g AO capacity values, respectively. Lowest AO capacity was shown by EA extracts. In general, best antioxidant response was observed with *P. longum* extracts followed by *T. cardifolia* and *B. variegata* extracts.

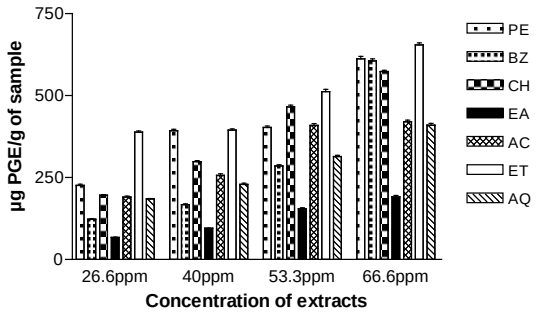

Figure 6. Antioxidant capacity determination of *P. longum* extracts by Phosphomolybdate method (Various components present in fruit samples were extracted with PE, BZ, CH, EA, AC, ET and AQ as described in methods section. The AO capacity was expressed as µg PGE/g of sample. Each value is represented as mean ± SEM of three replicates).

The antistaphylococcal activities of plant extracts are shown in Table 2. Several extracts showed inhibitory activity against *S. aureus.* Their inhibition zones ranged from 8-19 mm. The best inhibitory effect amongst three plants was exhibited by *T. cardifolia* extracts against test organism. Maximum zone of inhibition (ZOI) (19 mm) was obtained for EA extract at a concentration of 10 mg/disc. PE extracts of *T. cardifolia* and BZ extracts of *B. variegata* exhibited inhibitory potential at 5 mg/disc concentration. BZ and CH extracts derived from *T. cardifolia* produced significant bactericidal activity even at lower concentration of extracts (3.33 mg/disc) showing 11 mm ZOI. Low to moderate activity was observed with *P. longum* extracts (ZOI 8-12 mm) at 10 mg/disc concentration. *S. aureus* showed moderate susceptibility (ZOI 18 mm) to standard antibiotic vancomycin (30µg/disc). MBC was determined for extracts exhibiting comparatively better inhibitory efficacy against *S. aureus* (Table 3). The values ranged from 60.6 mg/ml to 0.43 mg/ml. The lowest MBC (0.43 mg/ml) was recorded for AC extract of *T. cardifolia* as well as for EA and AC extracts of *P. longum*.

DISCUSSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA (11).

Present work was undertaken to assess the antioxidant and microbicidal effects of *B. variegata* leaf, *T. cardifolia* stem and *P. longum* fruits extracts. The antioxidant activity may be directly correlated with the phenolic content present in various extracts (Table 1). The ethanolic extract of *B. variegata* and *P. longum* possessed high phenolic contents followed by the acetone and water extracts, respectively. These extracts (ET, AC, AQ extracts) showed higher antioxidant activity as compared to the other extracts (Fig. 1). In *T. cardifolia* extracts, the phenolic content is high in EA, AC, BZ and CH extracts while it is quite low in ET, AQ and PE extracts. Therefore differential antioxidant activity pattern (Fig. 2 and 5) observed in these extracts may find direct correlation between phenolic contents and the antioxidant activity (38).

Polyphenols, particularly flavonoids, which are widely distributed in the plant kingdom, and are present in considerable amount in fruits, vegetables, spices, medicinal herbs and beverages have been used to treat many human diseases, such as diabetes, cancer and coronary heart disease (6). Flavonoids have also been shown to exhibit the antioxidative, antiviral, antimicrobial and anti-platelet activities (26). The biological activities of these polyphenols in different systems are believed to be due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (10). Therefore, the relationships between the antioxidant activity (DPPH radical scavenging activity and total antioxidant capacity) and the content of the phenolic compounds were evaluated.

DPPH assay is one of the most commonly used methods for screening antioxidant activity of plant extracts (29). It has been largely used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts (23). DPPH produces violet colour in methanol solution. It is reduced to a yellow coloured product, diphenylpicryl hydrazine. Antioxidants react with DPPH, a nitrogen-centered free radical, which is a stable, and convert it to α, α-diphenyl-*β*-picryl hydrazine. The degree of discoloration indicates the scavenging potentials

Table 2. Inhibitory efficacy of extracts against *S. aureus*

Plants	PE	BZ	CH	EA	AC	ET	AQ
B. variegata	-	10*	-	-	8	8	-
T. cardifolia	12*	11^{+}	11^{+}	19	15	9	10
P. longum	8	-	-	12	12	10	-

Numbers represent ZOI (zone of inhibition) which are shown in mm. ZOI for vancomycin (30 µg/disc) was 18 mm. Experiments were performed in triplicate and values represent average. Normal disc contents 10 mg/disc. Asterics (*) and plus ($^{+}$) represent disc contents 5 mg/disc and 3.33 mg/disc, respectively. Abbreviations: PE - petroleum ether, BZ - benzene, CH - chloroform, EA - ethyl acetate, AC - acetone, ET - ethyl alcohol and AQ - water.

Table 3. Minimum bactericidal concentration (MBC) of potent extracts against *S. aureus* (mg/ml)

Plants	PE	BZ	EA	AC
B. variegata	nt	60.60	nt	nt
T. cardifolia	20.20	6.72	14.20	0.43
P. longum	nt	nt	0.43	0.43

Abbreviations: nt- not done, PE - petroleum ether, BZ - benzene, EA - ethyl acetate and AC – acetone.

of the antioxidant extracts (Fig. 1, 2, 3). The decrease in absorbance by the DPPH radical with increasing concentration of the extracts in dose dependent manner results in the rapid discolouration of the purple DPPH, suggesting that extracts of *B. variegata, T. cardifolia* and *P. longum* have radical scavenging antioxidant activity due to its proton donating ability (2). The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. Several extract fractions exhibited significantly higher inhibition percentage (stronger hydrogen –donating ability) which can be positively correlated with total phenolic content (44).

It is well-known that free radicals cause autoxidation of unsaturated lipids in food (42). On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid (22, 44). The data obtained in our experiments revealed that phytochemicals present in the extracts are primary antioxidants and act as free radical scavengers.

The phosphomolybdenum method is based on the reduction of molybdenum (VI) by the antioxidants and the formation of a green molybdenum (V) complex, which shows maximum absorbance at 695 nm (36). In our study, plant extracts exhibited varying degrees of antioxidant capacity (Fig. 4, 5, and 6). It is difficult to assign an order of antioxidant (AO) capacities to the extracts because of the differential responses. The order of AO capacity of *B. variegata* extracts was ET=CH>AC>PE>EA>BZ>AQ, while in case of *T. cardifolia* it was AC>ET>EA>CH>BZ > AQ>PE. The order of efficacy of *P. longum* extracts was ET>CH≈PE>BZ>AC>AQ>EA. The difference in AO capacity of different extracts may be attributed to differences in their chemical composition. Recent reports indicated that several bioactive compounds present in plants have strong antioxidant activity (13, 20). The antioxidant activity shown by our plant extracts could be attributed to the presence of flavonoids, polyphenols, and tannins (28).

The antioxidant activities of the individual phenolic compounds may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, and other structural features. Dihydroxylation in both rings and in the 3-postion in catechin, myricetin,

quercetin, and epicatechin is required for antioxidant activity as reported in various lipid systems (35). Although the phenolic compounds have similar chemical properties, their reduction capacity is not a very precise predictor of their antioxidant activity (44).

Because of emergence of multiple drug resistance in human pathogenic organisms, search for new antimicrobial substances from alternative sources including plants is gaining momentum (3). The plants studied in the current work exhibited substantial antibacterial activities (Table 2) as shown by low MBC values of extracts against the test organism *S. aureus* (Table 3). The best inhibitory activity was observed for EA, PE, BZ and AC fractions. Anti-staphylococcal activities of a few extracts were found comparable to the activity of vancomycin discs (30 µg/disc). Available reports tend to show that secondary metabolites such as alkaloids, flavonoids, tannins and other compounds of phenolic nature are responsible for the antimicrobial activities in higher plants (7, 25, 32). Monoterpenes, sesquiterpenes, alcohols and aldehydes (17, 46, 48) have been reported to exhibit antibacterial activity in spices against respiratory tract infections. Cyclic terpene compounds have been reported to cause loss of membrane integrity and dissipation of proton motive force (43). Therefore, presence of some of these phytochemicals along with phenolic compounds could to some extent justify the observed antistaphylococcal activities in the present study. Prominent antistaphylococcal activity in *T. cardifolia* extracts (ZOI 9-19 mm) could be attributed to the presence of variable amounts of bioactive secondary metabolites in plant samples. The composition of these secondary metabolites in turn varies from species to species, climatic conditions, and the physiological state of developments of the plants (16). It is possible that phytochemicals present in these medicinal plants may find their use as future antibacterial agents. However, the exact mechanism of antibacterial effects from *T. cardifolia*, *B. variegata* and *P. longum* needs to be further examined for potential uses.

Several extract of test plants exhibited strong antioxidant activity in the *in vitro* assays. *P. longum* extracts in DPPH, and total antioxidant capacity determination assays were found to be more effective than *T. cordifolia* and *B. variegata* extracts. In microbiological assays *T. cordifolia* extracts exhibited better antistaphylococcal activity than *P. longum* and *B. variegata* extracts. This indicates that the extracts from test plants or their derived phytochemicals have considerable potential to prevent diseases caused by the overproduction of radicals as well as diseases resulting from pathogenic bacteria. The results can provide useful insight for further applications of these phytoconstituents in future pharmaceutical preparations after performing *in vivo* clinical researches.

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Other articles in this theme issue include references (50-65).

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