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Antimicrobial, antioxidant, and synergistic properties of two nutraceutical plants: *Terminalia catappa* L. and *Colocasia esculenta* L.

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Abstract: Antibiotics have been effective in treating infectious diseases, but resistance to these drugs has led to the emergence of new and reemergence of old infectious diseases. Using a combination of plant extracts and antibiotics is one way of combating these multidrug-resistant microorganisms. The aim of this investigation was to evaluate the antimicrobial and antioxidant properties of 2 nutraceutical plants: *Terminalia catappa* and *Colocasia esculenta*. The antimicrobial activity of the plants was evaluated against 25 microorganisms by agar well diffusion method. *C. esculenta* did not show any antimicrobial activity against bacteria and fungi. Therefore, only methanol extract of *T. catappa* was selected for further study of synergistic activity with standard antibiotics. Maximum synergistic activity was seen against fungi as compared to individual extracts. The methanol extract of *T. catappa* showed good antioxidant and antimicrobial activity. The synergistic effect demonstrates the potential of this plant as a candidate for antibiotic-resistance–modifying compounds. The results suggest that the leaves of *T. catappa* are a good source of natural antioxidants and antimicrobics.

Key words: *Terminalia catappa*, *Colocasia esculenta*, synergism, antimicrobial activity, antioxidant activity, ABTS, DPPH, superoxide anion radical scavenging assay, ferric reducing antioxidant power

1. Introduction

Infectious diseases caused by bacteria and fungi affect millions of people worldwide. Treating bacterial infections with antibiotics is beneficial, but their indiscriminate use has led to an alarming rate of resistance among microorganisms (1). Multiple drug resistance in microbial pathogens is an ongoing global problem. This results in loss of effective antibiotics and loss of budget for infectious disease treatment. Thus, there is an urgent and constant need for exploration and development of cheaper, effective, new plant-based drugs with better bioactive potential and the fewest possible side effects. Hence, attention has been directed toward biologically active extracts and compounds from plant species to fight against microbial diseases (2–8), as well as against degenerative diseases caused by free radicals (9). A few studies on the combination of antibiotics with various plant extracts have been reported (10,11).

With the growing incidence of infections resistant to current antibiotics, an arsenal of new agents for supplementing current antibiotics is needed. The alternative is combination therapy, which is beneficial for patients with serious infections caused by drug-resistant pathogens (12,13). The synergistic effect of combining antibiotics with bioactive plant extracts against multidrug–resistant microorganisms can lead to new forms of treatment of infectious diseases. However, the selection of an appropriate combination requires an understanding of the potential for interaction between the antimicrobial agents. Synergistic therapy can be used to expand the antimicrobial spectrum, to prevent the emergence of resistant mutants, to minimize toxicity, to obtain synergistic antimicrobial activity, etc. (14).

Synergistic effects may be due to certain complex formations that become more effective in the inhibition of a particular species of microorganisms by inhibiting the cell wall synthesis, interfering with enzymes, or causing cell death. Sometimes the use of a single antibiotic does not produce the desired or effective inhibitory effects. To overcome this, a combination of drugs is often used, and their synergistic effect surpasses their individual performance. In this sense, a promising strategy to enhance and/or broaden the biological antimicrobial activities seems to be the combination of 2 or more compounds, although to date, a rational basis for the use of phytochemicals against pathogens has not been sufficiently explored (15).

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During normal metabolism, free radicals are generated continuously as reactive oxygen species (ROS) and reactive nitrogen species (RNS), including diverse reactive entities, namely superoxide ($O2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$), peroxynitrite ($^{\bullet}ONOO^{-}$), and nitric oxide ($NO^{\bullet}$) radicals, as well as nonfree radical species such as hydrogen peroxide (H_2O_2), nitrous acid (HNO_2), and hypochlorous acid (HOCl) (16). Free radicals are derived from 2 sources: endogenous sources (nutrient metabolism, aerobic respiration, stimulated polymorphonuclear leucocytes and macrophages, the aging process, action of peroxisomes, and activation of inflammatory cells) (17,18) and exogenous sources (environmental agents such as tobacco smoke, ionizing radiation, air pollution, organic solvents, or pesticides).

Excessive amounts of free radicals can be harmful to our bodies and may destroy many major biological macromolecules when overproduced during conditions such as excessive exercise, hypoxia, and antioxidant system failure (19). ROS can attack various substrates in the body and contribute to the development of chronic diseases such as cancer, autoimmune disorders, rheumatoid arthritis, cataracts, aging, cardiovascular and neurodegenerative diseases (20), and they may sometimes cause death.

Antioxidants are the substances that provide protection against oxidative damage (21). They may function as free radical scavengers, complexes of prooxidant metals, reducing agents, and quenchers of singlet oxygen formation. Antioxidants help organisms deal with oxidative stress and are well protected against toxic free radicals by enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT); small antioxidant molecules (uric acid, glutathione, albumin, protein –SH groups, bilirubin); and certain vitamins (ascorbic acid, α-tocopherol) as well as β-carotenoids (22), which have the capacity to neutralize free radicals acting in concert (23).

The use of several synthetic antioxidant compounds like butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, and tertiary butylhydroquinone has been restricted because of their low solubility, moderate antioxidant activity, toxicity, carcinogenic effects (24,25), and negative health effects. Thus, the interest in natural antioxidants has increased considerably.

Today, the ongoing emergence of multidrug-resistant bacteria and many diseases mainly caused by free radicals are serious global problems. Thus, new antimicrobials and novel approaches to combating these problems are urgently needed. Combination therapy is a new approach that may be helpful in treating multidrug-resistant bacteria and diseases caused by oxidative stress. The objective of this research was to investigate the antimicrobial and antioxidant potential of *Terminalia catappa* L. and *Colocasia esculenta* L. leaves, using a number of antioxidant models, as well as the antimicrobial activity against different microorganisms and their synergistic effects.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide reduced (NADH), gallic acid, ascorbic acid, $FeSO_4$, quercetin, Folin–Ciocalteu reagent, aluminum chloride, potassium acetate, ferric chloride, 2,4,6-tripyridyl-s-triazine (TPTZ), Tris–HCl, sodium acetate, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), nutrient broth, Sabouraud dextrose broth, Mueller Hinton agar no. 2, and Sabouraud dextrose agar were obtained from Hi-Media, Mumbai, India; petroleum ether, acetone, methanol, and so on were obtained from Merck, India.

2.2. Plant collection

The leaves of *T. catappa* L. (PSN291) and *C. esculenta* L. (PSN748) were collected in August 2010 from Rajkot, Gujarat, India, and identified by comparison with specimens available at the herbarium of the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves were washed thoroughly with tap water, shade-dried, homogenized to fine powder, and stored in airtight bottles.

2.3. Extraction

The dried powder of the 2 plant leaves was extracted individually by the cold percolation method (26) using different organic solvents like petroleum ether, acetone, and methanol. First, 10 g of dried powder was added to 100 mL of petroleum ether in a conical flask, which was plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 min. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 mL of solvent (acetone and methanol) in a conical flask, which was plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvents were evaporated; the dry extract was stored at 4 °C in airtight bottles. The residues were weighed to obtain the extraction yield.

2.4. Antimicrobial assays

2.4.1. Microorganisms tested

The microorganisms used were obtained from the National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4 °C. The gram-positive bacteria studied were *Staphylococcus aureus* ATCC25923,

Staphylococcus aureus ATCC29737, *Staphylococcus epidermidis* ATCC12228, *Staphylococcus albus* NCIM2178, *Bacillus megaterium* ATCC9885, *Bacillus subtilis* ATCC6633, *Bacillus cereus* ATCC11778, *Corynebacterium rubrum* ATCC14898, *Listeria monocytogenes* ATCC19112, and *Micrococcus flavus* ATCC10240. The gram-negative bacteria were *Proteus morganii* NCIM2040, *Proteus vulgaris* NCIM2857, *Enterobacter aerogenes* ATCC13048, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM2241, *Pseudomonas aeruginosa* ATCC27853, *Salmonella typhimurium* ATCC23564, *Citrobacter frundii* NCIM2489, *Klebsiella aerogenes* NCIM2098, and *Escherichia coli* NCIM2931. Yeasts were *Candida albicans* ATCC2091, *Candida neoformans* NCIM3542, *Candida glabrata* NCIM3448, *Candida epicola* NCIM3367, and *Trichosporon beigelii* NCIM3404.

2.4.2. Antibiotics used

The antibiotics used were 30 µg tetracycline (T^{30}) for bacteria and 100 units/disk nystatin (NS^{100}) and 100 units/disk amphotericin B (AP^{100}) for fungi.

2.4.3. Agar well diffusion assay

The test organism was activated by inoculating a loop full of the strain in 25 mL of nutrient broth/Sabouraud dextrose broth, which was kept overnight on a rotary shaker. Mueller Hinton agar and Sabouraud dextrose agar media were used for antibacterial and antifungal activity respectively. The assay was performed by the agar well diffusion method (27,28), with 200 µL of inoculum (1×10^8 cfu/mL) introduced into molten Mueller Hinton agar/Sabouraud dextrose agar and poured into petri dishes when the temperature reached 40–42 °C. The media were solidified and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). Next, 100 µL of the test drug (20 mg/mL and 10 mg/mL in dimethyl sulfoxide [DMSO]) was introduced into the well and the plates were incubated at 37 and 28 °C for 24 and 48 h for bacteria and fungi, respectively. DMSO was used as a negative control. All the tests were performed in triplicate under strict aseptic conditions. The microbial growth was determined by measuring the diameter of the zone of inhibition in millimeters.

2.4.4. Disk diffusion assay

Antimicrobial activity of the methanolic extract of *T. catappa* with standard antibiotics was assessed against 10 gram-positive bacteria, 10 gram-negative bacteria, and 5 fungi by using the agar disk diffusion method (5,29). The petri dishes were prepared by pouring 20 mL of sterilized molten Mueller Hinton agar for bacteria and Sabouraud dextrose agar for fungal strains, which was then seeded with 200 µL of test culture containing 1×10^8 cfu/mL for bacteria and 2.0×10^5 spores/mL for fungal strains as McFarland 0.5 turbidity standard. The media were allowed to solidify. Sterile filter paper disks (6 mm) were impregnated with 20 µL of each drug separately and allowed to saturate for 30 min, and were then were placed on the surface of the agar plates, which had previously been inoculated with test microorganisms. All plates were incubated for 24 and 48 hours at 37 °C and 28 °C for the bacteria and fungal strains, respectively. Results were recorded by measuring the zone of inhibition appearing around the disks. All the tests were performed in triplicate and the mean values are presented. Pure DMSO was used as the negative control.

2.5. Antioxidant testing assays

2.5.1. DPPH free radical scavenging assay

The free radical scavenging activity was measured using DPPH following the method of McCune and Johns (30) with modifications. The reaction mixture, consisting of DPPH in methanol (0.3 mM, 1 mL), 1 mL methanol, and different concentrations of the solvent extracts (1 mL), was incubated for 10 min in the dark, after which the absorbance was measured at 517 nm. Ascorbic acid was used as the positive control (31). The percentage of inhibition was determined by comparing the results of the test and the control. Percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - (A/B)] \times 100,$$

where B is the absorbance of the blank (DPPH, plus methanol) and A is absorbance of the sample (DPPH, methanol, plus sample).

2.5.2. Determination of $ABTS^{\bullet+}$ radical scavenging activity

The ABTS radical cation ($ABTS^{\bullet+}$) decoloration test is a spectrophotometric method widely used for assessment of the antioxidant activity of various substances. The $ABTS^{\bullet+}$ scavenging activity was measured using the method of Re et al. (32). ABTS was dissolved in water to a concentration of 7 mM. $ABTS^{\bullet+}$ was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration), allowing the mixture to stand in the dark at room temperature for 16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. The $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of 0.850 ± 0.05 at 734 nm, and 3.0 mL of this $ABTS^{\bullet+}$ solution was added to 1.0 mL of different concentrations of the extract and incubated for 4 min at room temperature. Absorbance was measured spectrophotometrically at 734 nm. For the control, 1.0 mL of methanol was used in place of extract. Ascorbic acid was used as a positive control (33). The radical scavenging activity of the tested samples is expressed as the IC_{50} value. The percentage of inhibition was calculated using the following formula:

Inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$,

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample and the standard compound.

2.5.3. Superoxide anion radical scavenging assay

The superoxide anion radical scavenging activity was measured according to the procedure described by Robak and Gryglewski (34). In the PMS/NADH–NBT system, the superoxide anion derived from dissolved oxygen from the PMS/NADH coupling reaction reduces NBT. The reaction mixture, in a 3-mL final volume, consisted of 0.5 mL of Tris–HCl buffer (16 mM, pH 8.0), 0.5 mL of NBT (0.3 mM), 0.5 mL of NADH (0.936 mM) solution, and 1.0 mL of various concentrations of different solvent extracts. The reaction was initiated by adding 0.5 mL of PMS solution (0.12 mM) to the mixture. The reaction mixture was then incubated at 25 °C for 5 min and the absorbance was measured at 560 nm against a blank sample. Gallic acid was used as a positive control (34). The assay was carried out in triplicate, and the mean values ± standard errors of mean (SEMs) are presented. The percentage inhibition was determined by comparing the results of the test and the control.

2.6. Ferric reducing antioxidant power assay

The reducing ability of the sample was determined by the ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (35). The FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH: 3.6). To perform the assay, 0.1 mL of the extract is added to 3.0 mL of FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ in 40 mM hydrochloric acid, and 1 part 20 mM $FeCl_3$), and the reaction mixture is incubated at 37 °C for 10 min. Absorbance was measured at 593 nm. $FeSO_4$ was used as a positive control. The antioxidant capacity is based on the ability to reduce the ferric ions of the sample, and it is calculated from the linear calibration curve and expressed as mM $FeSO_4$ equivalents per gram of sample. The assay was carried out in triplicate, and the means ± SEMs are presented.

2.7. Quantitative phytochemical analysis

2.7.1. Determination of total phenol content

The total phenol content was determined according to the Folin–Ciocalteu reagent method (36). First, 0.5 mL of extract and 0.1 mL of 0.5 N Folin–Ciocalteu reagent were mixed, and the mixture was incubated at room temperature for 15 min. Next, 2.5 mL of saturated sodium carbonate solution was added, and the mixture was further incubated for 30 min at room temperature; the absorbance was measured at 760 nm. Gallic acid was used as a positive control (37). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound). The assay was carried out in triplicate, and mean values ± SEMs are presented.

2.7.2. Determination of flavonoid content

The flavonoid content was determined by the aluminum chloride colorimetric method (38). The reaction mixture, in a 3-mL final volume, consisted of 1.0 mL of sample (1 mg/mL), 1.0 mL of methanol, 0.5 mL of 1.2% aluminum chloride, and 0.5 mL of 120 mM potassium acetate, and it was incubated at room temperature for 30 min. The absorbance of all the samples was measured at 415 nm. Quercetin was used as the positive control (39,40). Flavonoid content is expressed in terms of quercetin equivalent (mg g^{-1} of extracted compound).

2.8. Statistical analysis

All experiments were repeated at least 3 times. Results were reported as means ± SEMs.

3. Results and discussion

3.1. Antimicrobial activity

The use of medicinal plants plays a vital role in covering the basic health needs of many and also produces a large number of secondary metabolites with antimicrobial effects on pathogens (41,42). All parts of plants individually or in combination show antimicrobial properties. A significant part of the chemical diversity produced by plants protects them against microbial pathogens. The antimicrobial activity of *T. catappa* and *C. esculenta* in 3 solvents was evaluated against 10 gram-positive bacteria, 10 gram-negative bacteria, and 5 fungal strains. The 3 solvent extracts of *C. esculenta* did not show any antimicrobial activity against the tested microorganisms (bacteria and fungi); hence, only the results of the antimicrobial activity of *T. catappa* are presented (Figure 1).

The petroleum extract did not show any activity against gram-positive bacteria but was active against 3 gram-negative bacteria (Figure 1a). The acetone extract showed activity against 3 gram-positive bacteria; maximum activity was against *M. flavus* at 20 mg/mL drug concentration (Figure 1b). The acetone extract showed activity against all the tested gram-negative bacteria; maximum activity was against *P. vulgaris*, followed by *K. aerogenes* and *P. morganii* at 20 mg/mL drug concentration (Figure 1c).

The methanol extract showed activity against all the tested gram-positive bacteria except *B. subtilis* at both concentrations and against *B. megaterium* and *S. epidermidis* at 10 and 20 mg/mL drug concentrations, respectively; maximum activity was against *M. flavus* (Figure 1d). The methanolic extract showed maximum activity against all the gram-negative bacteria, and the highest activity was against *P. vulgaris* (Figure 1e). Among all the solvent extracts, maximum activity was found in methanol extract; therefore, it was further used for study of synergistic activity with standard antibiotics.

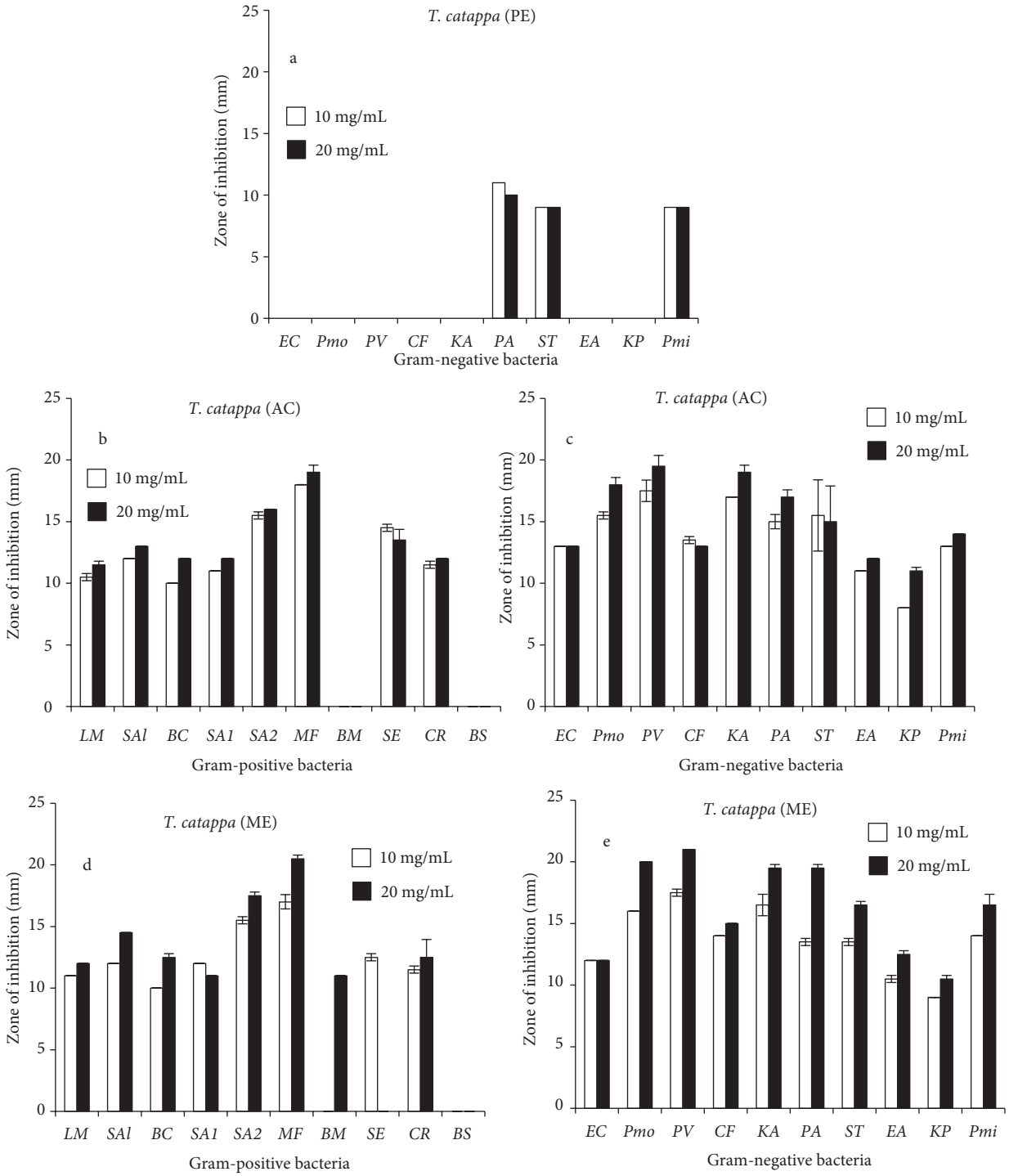

Figure 1. Antimicrobial activity of different solvent extracts of *T. catappa*: a) petroleum extract with gram-negative bacteria, b) acetone extract with gram-positive bacteria, c) acetone extract with gram-negative bacteria, d) methanol extract with gram-positive bacteria, e) methanol with gram-negative bacteria. Gram-positive bacteria: LM, *Listeria monocytogenes*; SAl, *Staphylococcus albus*; BC, *Bacillus cereus*; SA1, *Staphylococcus aureus* ATCC25923; SA2, *Staphylococcus aureus* ATCC29737; MF, *Micrococcus flavus*; BM, *Bacillus megaterium*; SE, *Staphylococcus epidermidis*; CR, *Corynebacterium rubrum*; BS, *Bacillus subtilis*. Gram-negative bacteria: EC, *Escherichia coli*; Pmo, *Proteus morganii*; PV, *Proteus vulgaris*; CF, *Citrobacter frundii*; KA, *Klebsiella aerogenes*; PA, *Pseudomonas aeruginosa*; ST, *Salmonella typhimurium*; EA, *Enterobacter aerogenes*; KP, *Klebsiella pneumoniae*; Pmi, *Proteus mirabilis*.

Sometimes the use of a single antibiotic does not produce the desired or effective inhibitory effect, and to overcome this, treatment with a combination of drugs may be attempted; their synergistic effect often surpasses their individual performance. In this study, the synergistic effect resulting from the combination of standard antibiotic with crude plant extract was verified against all tested microorganisms. The inhibitory effect of tetracycline was evaluated individually and in combination with the methanolic extract of *T. catappa* leaves.

Synergistic activity of methanolic extract of *T. catappa* leaves with tetracycline against gram-positive bacteria is shown in Table 1. Out of 10 different gram-positive bacteria tested, maximum synergistic activity was seen against 7 strains in the following order: *Staphylococcus epidermidis* > *Micrococcus flavus* > *Listeria monocytogenes* > *Bacillus subtilis* > *Corynebacterium rubrum* > *Bacillus cereus* > *Staphylococcus aureus*. *Staphylococcus epidermidis* was the most sensitive strain amongst all the tested gram-positive bacteria, and more synergism was observed with the methanolic extract of *T. catappa* and tetracycline as compared to individual action against *S. epidermidis*. Indifferent effect was found against *Staphylococcus albus* and *Bacillus megaterium*, while antagonistic effect was found against *Staphylococcus aureus* ATCC25923. Synergistic activity of the methanolic extract of *T. catappa* leaves with tetracycline against gram-negative bacteria is shown in Table 2. Out of 10 different gram-negative bacterial strains tested, maximum synergistic activity was found against 4 strains in the following order: *Proteus mirabilis* > *Escherichia coli* > *Pseudomonas aeruginosa* > *Enterobacter aerogenes*. Indifferent effect was observed against the remaining bacterial strains. Synergistic activity of the methanolic extract of *T. catappa* leaves with nystatin and amphotericin against fungi (yeast) is shown in Table 3. The synergistic effect was observed against all the tested fungi. Maximum synergism was seen in the methanolic extract of *T. catappa* leaves with nystatin and amphotericin-B against *Candida epicola*.

Antibacterial activity of the methanolic extract of *T. catappa* leaves with tetracycline showed the maximum synergistic effect against gram-positive bacteria (70%) as compared to gram-negative bacteria (40%). In antifungal activity, 100% synergism was seen in *T. catappa* leaves with nystatin and amphotericin-B against all the tested fungi, but maximum synergism was found against gram-positive bacteria in the methanolic extract of *T. catappa* leaves with tetracycline. There are many reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (43,44). This difference may be due to the structural differences in the cell walls of these bacteria. The gram-negative cell wall (made up of lipopolysaccharide) is a complex and multilayered structure, which makes access to the membrane more restricted and serves as a barrier to many environmental substances including synthetic and natural antibiotics. The gram-positive bacteria contain a single outer peptidoglycan layer, which is not an effective permeability barrier.

3.2. Antioxidant testing assays

There are many methods used to evaluate the free radical scavenging activity of compounds (45). The antioxidant activities of plant extracts vary with assay methods because

Table 1. Synergistic activity of methanolic extract of *Terminalia catappa* leaves and tetracycline against gram-positive bacteria.

No.	Microorganisms	Zone of inhibition (mm)			Effect
		T*	E*	TE*	
1	*Staphylococcus aureus* ATCC25923	19 ± 0.00	12 ± 0.29	18 ± 0.00	A
2	*Staphylococcus aureus* ATCC29737	22 ± 0.58	8 ± 0.00	30 ± 0.00	S
3	*Staphylococcus epidermidis* ATCC12228	0 ± 0.00	12 ± 0.00	28.5 ± 0.29	S
4	*Staphylococcus albus* NCIM2178	21 ± 0.00	7 ± 0.00	24.5 ± 0.87	ID
5	*Bacillus megaterium* ATCC9885	19 ± 0.00	9 ± 0.58	24.5 ± 0.29	ID
6	*Bacillus subtilis* ATCC6633	20 ± 0.00	0 ± 0.00	28 ± 0.00	S
7	*Bacillus cereus* ATCC11778	18 ± 0.29	0 ± 0.00	21.5 ± 0.29	S
8	*Corynebacterium rubrum* ATCC14898	0 ± 0.00	12 ± 0.00	18 ± 0.00	S
9	*Listeria monocytogenes* ATCC19112	0 ± 0.00	8 ± 0.00	22 ± 0.58	S
10	*Micrococcus flavus* ATCC10240	0 ± 0.00	14.5	29 ± 0.58	S

T = tetracycline; E = methanolic extract of *T. catappa*; TO = tetracycline + methanolic extract of *T. catappa*; S = synergistic effect; ID = indifference; A = antagonism; n = 3.
* The values are means ± SEMs (n = 3).

Table 2. Synergistic activity of methanolic extract of *Terminalia catappa* leaves and tetracycline against gram-negative bacteria.

No.	Microorganisms	Zone of inhibition (mm)			Effect
		T*	E*	TE*	
1	*Proteus morganii* NCIM2040	22 ± 0.00	11 ± 0.00	25 ± 0.00	ID
2	*Proteus vulgaris* NCIM2857	17 ± 0.58	11 ± 0.00	20 ± 0.00	ID
3	*Enterobacter aerogenes* ATCC13048	25 ± 0.29	0 ± 0.00	27.5 ± 0.29	S
4	*Klebsiella pneumoniae* NCIM2719	24 ± 0.00	12 ± 0.00	25 ± 0.00	ID
5	*Proteus mirabilis* NCIM2241	8 ± 0.00	9 ± 0.00	25 ± 0.00	S
6	*Pseudomonas aeruginosa* ATCC27853	8 ± 0.00	9 ± 0.00	21 ± 0.00	S
7	*Salmonella typhimurium* ATCC23564	19 ± 0.29	8 ± 0.00	23 ± 1.15	ID
8	*Citrobacter frundii* NCIM2489	20 ± 0.00	9 ± 0.29	26 ± 0.00	ID
9	*Klebsiella aerogenes* NCIM2098	18 ± 0.00	10 ± 0.00	21 ± 0.00	ID
10	*Escherichia coli* NCIM2931	16 ± 0.58	0 ± 0.00	21 ± 0.58	S

T = tetracycline; E = methanolic extract of *T. catappa*; TO = tetracycline + methanolic extract of *T. catappa*; S = synergistic effect; ID = indifference; A = antagonism; n = 3.
* The values are means ± SEMs (n = 3).

of the complex nature of phytochemicals present in them, the solvent used for extraction, etc. (46–49). It is thus important that several analytical methods and different substrates are used for evaluating the effectiveness of antioxidants. The methods selected were those most commonly used for the determination of antioxidant activities of plant extracts. In the present study, DPPH, superoxide anion and ABTS radical scavenging activity, and FRAP were evaluated in *T. catappa* and *C. esculenta* leaves extracted in acetone and methanol.

3.2.1. DPPH free radical scavenging activity

The free radical scavenging activities of acetone and methanol extracts of leaves of *T. catappa* and *C. esculenta* were tested by the DPPH method. The IC_{50} values of DPPH free radical scavenging activity are shown in Table 4. Both extracts of *C. esculenta* showed an IC_{50} value greater than 1000 µg/mL. The acetone extract of *T. catappa* leaves showed an IC_{50} value of 28.5 µg/mL, while the methanol extract showed maximum scavenging activity with a low IC_{50} value (23 µg/mL), which was near that of standard ascorbic acid (IC_{50} = 11.4 µg/mL) (Table 4).

3.2.2. ABTS radical scavenging activity

The ABTS radical cation scavenging activities of *T. catappa* and *C. esculenta* leaves and their synergistic activities are shown in Table 4. The activity was concentration-dependent, and the maximum scavenging activity was found in the methanolic extract of *T. catappa* leaves (IC_{50} = 8.25 µg/mL), which was near that of standard ascorbic acid (IC_{50} = 6.5 µg/mL), followed by acetone extract (IC_{50} = 10.25 µg/mL). In *C. esculenta*, acetone extract

Table 3. Synergistic activity of methanolic extract of *Terminalia catappa* leaves with nystatin and amphotericin-B against fungi.

No.	Microorganisms	Zone of inhibition (mm)			Effect	Zone of inhibition (mm)			Effect
		NS*	E*	NSE*		AP*	E*	APE*	
1	*Candida albicans* ATCC2091	22 ± 0.00	0 ± 0.00	25 ± 0.00	S	112 ± 0.00	0 ± 0.00	15 ± 0.00	S
2	*Candida neoformans* NCIM3542	26 ± 0.29	0 ± 0.00	29 ± 0.58	S	13 ± 0.29	0 ± 0.00	18 ± 0.00	S
3	*Candida glabrata* NCIM3448	23 ± 0.00	0 ± 0.00	25.5 ± 0.29	S	11 ± 0.00	0 ± 0.00	14 ± 0.00	S
4	*Candida epicola* NCIM3367	22 ± 0.29	0 ± 0.00	28 ± 0.58	S	16 ± 0.58	0 ± 0.00	23 ± 0.00	S
5	*Trichosporon beigelii* NCIM3404	19 ± 0.00	0 ± 0.00	21 ± 0.00	S	12 ± 0.00	0 ± 0.00	15 ± 0.00	S

NS = nystatin; AP = amphotericin B; E = methanolic extract of *T. catappa*; NSE = nystatin + methanolic extract of *T. catappa*; APE = amphotericin B + methanolic extract of *T. catappa*; S = synergistic effect; ID = indifference; A = antagonism; n = 3.
* The values are means ± SEMs (n = 3).

Table 4. DPPH free radical, ABTS radical activity, and superoxide anion radical scavenging activities; ferric reducing antioxidant power; and total phenol and flavonoid contents of 2 plant solvent extracts.

Plant name	IC_{50} values (µg/mL)						FRAP (M/g)		Total phenol content (mg/g)*		Total flavonoid content (mg/g)*	
	DPPH		ABTS		SO							
	AC	ME	AC	ME	AC	ME	AC	ME	AC	ME	AC	ME
Terminalia catappa	28.5	23	10.25	8.25	174	140	37.12 ± 0.18	49 ± 1.08	201 ± 0.14	206 ± 0.19	10 ± 0.00	36 ± 0.00
Colocasia esculenta	>1000	>1000	172.5	186	>1000	>1000	NA	1 ± 0.02	8 ± 0.36	27 ± 0.55	32 ± 2.00	11 ± 1.00
T. catappa + *C. esculenta* (ME 1:1)	ND	ND	ND	15.5	ND	270	ND	ND	ND	ND	ND	27.69 ± 0.30
Standard	11.4 Ascorbic acid		6.5 Ascorbic acid		185 Gallic acid		Equivalent to $FeSO_4$		Equivalent to gallic acid		Equivalent to quercetin	

NA = No activity; ND = not done. *The values are means ± SEMs (n = 3).

showed more scavenging activity than methanol extract. In order to evaluate the synergistic effects of these plants on ABTS cation scavenging activity, the methanol extract of *T. catappa* was mixed with the methanol extract of *C. esculenta* in a ratio of 1:1; the combination gave maximum synergism as compared to each individually. The IC_{50} value of this combined extract was 15.5 µg/mL (Table 4). Thus, *C. esculenta* showed 12-fold reduction in IC_{50} value on synergism with *T. catappa*, while *T. catappa* had a 2-fold increase in IC_{50}. This result is very promising because it indicates a reduction in the concentration of the drug used in combination for treating various diseases and disorders.

3.2.3. Superoxide anion radical scavenging activity

Results of the superoxide anion radical scavenging activity of *T. catappa* and *C. esculenta* leaves and their synergism are given in Table 4. The IC_{50} value of the methanol extract of *T. catappa* was 140 µg/mL, while that of the acetone extract was 174 µg/mL. Both extracts appeared to be better scavengers of superoxide radicals than standard gallic acid (IC_{50} = 185 µg/mL). The acetone and methanol extracts of *C. esculenta* showed poor superoxide anion radical scavenging activity (>1000 µg/mL, Table 4), but in synergy with *T. catappa* (1:1 ratio), they showed maximum scavenging activity compared to each individually (IC_{50} = 270 µg/mL). Individually, *C. esculenta* had an IC_{50} value of >1000 µg/mL, but in synergy, the IC_{50} value decreased 2-fold.

3.3. **FRAP assay**

The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue-colored complex of ferrous ion (Fe^{2+}) and TPTZ. Prior to this, a colorless ferric ion (Fe^{3+}) gets oxidized to ferrous ion (Fe^{2+}) by the action of electron-donating antioxidants (50). The results showed that the methanol extract of *T. catappa* (49 M/g) had the highest antioxidant capacity by the FRAP method, followed by acetone extract (37.12 M/g), which was equivalent to $FeSO_4$ (Table 4). The acetone extract of *C. esculenta* did not show any activity, while the methanol extract showed activity with a value of 1 M/g. This result proved that the methanol extract of *T. catappa* leaves was able to reduce the complex ferric ion (Fe^{3+}) TPTZ to another complex ferrous ion (Fe^{2+}) TPTZ by releasing an electron.

3.4. Quantitative phytochemical analysis

Plant secondary metabolites such as flavonoids, phenolic acids, and polysaccharides belong to the nonenzymatic antioxidants. These compounds play many physiological roles in plants and some of them are also favorable to human health due to their high redox potential. They also exhibit a wide range of biological activity such as antimicrobial activity, anticarcinogenicity, and antiproliferation (51,52). The maximum phenol and flavonoid content was in the methanol extract of *T. catappa* (Table 4). The methanol extract of *T. catappa* also showed good antioxidant activity, thus supporting the general view that phenolic content and antioxidant activity have a direct correlation and that the phenolic content of a plant is a good indicator of its antioxidant capacity (47).

4. Conclusion

In the present investigation, the methanolic extract of *T. catappa* showed good antimicrobial activity individually and in combination with standard antibiotics, i.e. it showed a good synergistic activity. It also showed good antioxidant activity. The synergistic effect of methanolic extract of *T. catappa* leaves and standard antibiotics demonstrates the potential of this plant as a candidate for antibiotic-resistance–modifying compounds. Hence, this plant warrants further study. Fractionation of this extract, and isolation and identification of the compounds responsible for the synergism, are in progress. An elucidation of the mechanism of action of the compounds to determine the therapeutic applicability of such compounds in combination therapy is a subject in need of further study.

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