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Research Article

FREE RADICAL SCAVENGING AND ANTIMICROBIAL POTENTIAL OF MIXTURE OF SELECTIVE MEDICINAL PLANTS

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ABSTRACT

Objectives: Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. The present study was aimed to evaluate antioxidant, antimicrobial activity and phytochemical screening of the mixture of plants extracts.

Materials and Methods: The antimicrobial activity of the mixture was tested by agar well diffusion assay and antioxidant or free radical scavenging activity of the mixture was assessed against 1,1-diphenyl-2-picrylhydazyl (DPPH) and ferric reducing agent. Total phenolic content and phytochemical screening was also determined to assess their corresponding effect on the antioxidant activity of the plants mixture.

Results: Among all the four extracts (viz. methanol, ethanol, ethyl acetate and benzene) of the mixture, methanol and ethanol extract exhibited maximum activity against *Lactobacillus acidophilus* and *Staphylococcus aureus*, respectively. Benzene and ethyl acetate extracts of mixture was found to be significantly effective against all fungal strains tested with the inhibition zone ranging from 26.0±1.41 to 11.0±0.43 mm. Ethyl acetate and benzene extract of the mixture were highly effective against *Aspergillus terreus* (20.5±0.71 mm) and *Aspergillus niger* (26±1.41 mm) respectively. The superoxide radical scavenging activity (as evaluated by DPPH assay) of ethanol extract of the mixture at concentration of 100 µg/ml was found to be 74.54% followed by methanol, i.e., 72.10% whereas maximum metal chelating activity was observed in ethyl acetate (76.82%) followed by methanol extract (72.91%) and ethanol extract (62.11%) respectively. The IC_{50} value of the ethanol extract of the mixture was more than that of L-ascorbic acid showing the maximum inhibitory effect followed by methanol extract as evaluated by DPPH assay while the same was higher in ethyl acetate extract as evaluated by ferric reducing agents.

Conclusion: These finding provides evidence that plant mixture has strong antimicrobial and antioxidant effect and hence may be used as a source of natural antibiotics for the treatment of the diseases caused by the above pathogenic micro-organisms.

Keywords: Plants mixture extracts, Antimicrobial activity, Antioxidant activity, Minimum inhibitory concentration, Phytochemical screening.

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1].

The problem of microbial resistance is increasing at a faster rate, and the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken in order to reduce this problem, for example, to control the use of antibiotic, development of research for better understanding of genetic mechanisms for resistance and to continue studies to develop new drugs (either synthetic or natural). The problem of antibiotic resistance has negative impacts on antibiotic therapy. Hence, successful empiric therapy is much more difficult to be achieved. The emergence of drug resistance is an evolutionary process which is based on the selection of organisms that have an enhanced ability to survive and reproduce even in the presence of a drug [2]. Despite the existence of conventional antimicrobial agents, resistant or multi-resistant strains of pathogenic micro-organisms are continuously appearing [3]. This imposes the need to search for new effective drugs to overcome the problem.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies of natural therapies. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated for better understanding of their properties, safety and efficiency [4]. The use of plant extracts and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments.

Many plants have been used because of their antimicrobial activity. This activity is due to synthesis of secondary metabolites in plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils [5], as well as in tannin [6]. Studies of plant extract (*Thuja orientalis* and *Argemone mexicana*) suggest that these are potent antimicrobials against pathogenic bacteria and fungi and are acceptable with respect to the standard antibiotics and fungicides. Hence, synthetic antibiotics can be replaced with plant extract based antimicrobial medicines [7-11].

Medicinal plants constitute one of the main sources of new pharmaceuticals and health care products. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents [12]. The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins [13], carotenoids [14], flavonoids [15] and other phenolic compounds. In this study, the antimicrobial and antioxidant effect of the mixture of eight medicinal plants was carried out against selected pathogens.

MATERIALS AND METHODS

Collection of plant material

Plants (*Phyllanthus emblica*, *Semecarpus anacardium* Linn. *Zingiber officinale*, *Terminalia bellirica*, *Tinospora cordifolia*, *Terminalia chebula*,

Acorus calamus, *Embelia ribes*) were collected from a pansari shop in the local market of Sirsa. The various plants were brought to the laboratory. They were shade dried and grinded separately. The powder was stored in airtight containers at room temperature.

Microbial strains

The micro-organisms used in this study were *Staphylococcus aureus* MTCC 87, *Bacillus cereus* MTCC 6728, *Pseudomonas fluorescens* MTCC 7200, *Candida albicans* MTCC 854, *Salmonella typhi*, *Aeromonas hydrophila*, *Shigella*, *Lactobacillus acidophilus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* were taken from the department of biotechnology, CDLU, Sirsa. Strains of *S. typhi* were also isolated from infected human blood.

Isolation of *S. typhi* from field samples

S. typhi was isolated from blood samples of the patients suffering from typhoid. Three blood samples were collected from civil hospital Fatehabad (Haryana). Isolation was carried out by serial dilution method. Then 1.0 ml of blood from each dilution was poured in different well labeled sterilized Petri-plates. Molten bismuth sulphite agar media was poured in the plates and allowed to solidify. After solidification of the media, plates were kept in an incubator for 48 hr at 37°C. After 2 days, small black color colonies appeared on plates which were transferred to the liquid nutrient broth medium.

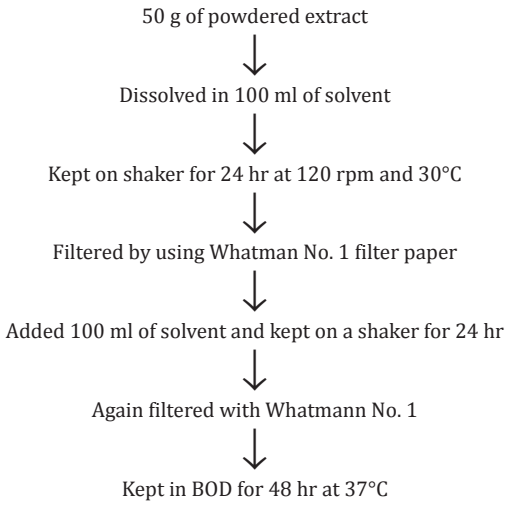
Preparation of plant extracts

Extract was prepared by mixing dry and grounded powder of different medicinal plants in the following amount.

Plants

P. emblica	10.0 g
S. anacardium Linn	05.0 g
Z. officinale	10.0 g
T. bellirica	10.0 g
T. cordifolia	07.5 g
T. chebula	10.0 g
A. calamus	07.5 g
E. ribes	10.0 g

Powder of all these plants was mixed properly and from this mixture, 50 g was taken. Extracts were prepared in four solvents, i.e., methanol, ethanol, benzene and ethyl acetate.



Antimicrobial activity

Agar well diffusion assay described by Perez [16] was used for testing antibacterial and antifungal activity. Hundred µl suspension of 24 hr old culture of test organism was inoculated on the agar plates and spread on to the surface of the agar with the help of a sterilized glass spreader. After 5 minutes of inoculation of test micro-organism, wells (2.5 mm diameter) were prepared with the help of sterilized steel cork borer. A concentration of 0.5 g/ml of plant extract was taken from the stock solution for agar well diffusion assay. Wells of each plate were loaded with 60 µl of crude plant extracts. Dimethyl sulfoxide was used as a negative control while ciprofloxacin, ampicillin and fluconazole were used as a positive control. The plates were then aerobically incubated at 30±2°C for *S. aureus*, at 37±2°C for 24 hr for other bacterial strains and at 28±2°C for 72 hr for fungal test organisms.

Minimum Inhibitory Concentration (MIC) of the extracts of the mixture

The MIC of each extracts of the plant mixture showing antimicrobial activity was evaluated by broth dilution method. Nutrient broth was prepared according to the manufacturer's instructions. This test was performed with five different concentrations (100, 50, 25, 12.5, 6.25 µg/ml) of each extracts. Serial dilution of the extract was carried out with nutrient broth to give above concentrations. 0.1 ml of the each micro-organism was inoculated into the dilutions and incubated at the above mentioned temperatures for 24 hr. After 24 hr absorbance was taken at 405 nm.

Antioxidant activity

1,1-diphenyl-2-picrylhydazyl (DPPH) radical scavenging assay

The DPPH free radicals scavenging activity of all the four extracts (ethanol, methanol, benzene, ethyl acetate) of plant mixture was determined according to the method given by Bhanja *et al*., [17]. One mg of extract powder was dissolved in 1.0 ml of 50 % methanol solution to obtain 1000 µg/ml sample solution. This solution was serially diluted into 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml with 50% methanol. In test tube 1.0 ml DPPH (0.1 mM), 0.45 ml of Tris-HCl buffer (50 mM and pH 7.4) and 0.05 ml samples was taken and kept at room temperature for 30 minutes. The reduction of the DPPH free radical was measured at 515 nm. DPPH is a purple-colored stable free radical; when reduced it becomes the yellow-colored diphenylpicrylhydrazine. L-ascorbic acid was used as a positive control. The antioxidant activity of test samples was evaluated by calculating the percent inhibition of superoxide anion radicals by applying the following formula.

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control (blank, without the extract) and A_1 was the absorbance of the extract. The antioxidant activity of each sample was expressed in terms of IC_{50}.

Chelating effect on ferrous ions

The chelating effect on ferrous ions was determined according to the method of Dinis *et al*. [18]. Each extract with different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml) was added in 2.0 ml methanol. To this mixture 0.1 mM $FeCl_2$ and 0.2 ml of 5.0 mM ferrozine solutions was added. After reaction for 10 minutes, absorbance was measured at 562 nm. A lower absorbance indicates a stronger chelating ability. Butylated hydroxytoluene (BHT) was used as control. The percentage of chelating effect of ferrous ion of test samples was evaluated by percent inhibition of superoxide anion radicals by applying following formula.

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control (blank, without the extract) and A_1 was the absorbance of the extract. The percentage of chelating effect of ferrous ion of test samples was determined and compared with BHT, which was used as a standard (positive control).

Total Phenolic Content (TPC) (Folin–Ciocalteu Method)

The amount of total phenolics in extracts was determined according to the Folin–Ciocalteu method. Extract powder (1.0 mg) was dissolved

in 1.0 ml of Folin–Ciocalteu's reagent and 0.8 ml of sodium carbonate (7.5%). The contents in the tube were mixed and allowed to stand for 30 minutes. Absorbance was measured at 765 nm. The TPC was expressed as gallic acid equivalents (GAE) in mg/g sample.

Phytochemical screening
The phytochemical analysis of the crude methanol, ethanol, ethyl acetate and benzene extracts of the mixture was carried out in order to ascertain the presence of the constituents such as sterol, tennins, flavonoids, alkaloids, sugar, protein, saponins, tannins, etc. by utilizing standard methods of analysis [19,20].

Test for sterols (Salkowaski Reaction)
Few mg of each plant extract was dissolved in 2.0 ml chloroform and then 2.0 ml of concentraed sulphuric acid (H_2SO_4) was added from the side of the test tube. Test tube was shaken for few minutes. Red color development in layer of chloroform indicated the presence of sterols.

Test for tannins (Ferric Chloride Reagent Test)
The test sample of each extract was taken separately in water, warmed and filtered. To a small volume of this filtrate, a few drops of 5% (w/v) solution of ferric chloride prepared in 90% alcohol were added. Dark green or deep blue color indicated the presence of tannins.

Test for protein (Xanthoproteic Test)
The extract (few mg) was dissolved in 2.0 ml water and then 0.5 ml of concentrated nitric acid was added in it. Yellow color indicated the presence of proteins.

Test for sugars (Fehling's Test for Free Reducing Sugar)
Fehling's solution was prepared as follows:

Solution A	
$CuSO_4$	34.64 g
H_2SO_4	0.50 ml
Distilled water	500.00 ml
Solution B	
Sodium potassium tartarate	176.0 g
NaOH	77.0 g
Distilled water	500.00 ml

The two solutions were mixed in equal volumes immediately before use.

About 0.5 g each extract was dissolved in distilled water and filtered. The filtrate was heated with 5.0 ml of equal volume of Fehling's solution A and B. Formation of red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Test for alkaloids (Wagner's Test)
Sample was mixed with 5.0 ml of 1.5% aqueous HCl and filtered. These filtrates were then used for testing alkaloids with the following reagent.

1.27 g of iodine and 2.0 g of potassium iodide were dissolved in 5.0 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to test filtrate, a brown flocculent ppt. was formed indicating the presence of alkaloids in the test sample.

Test for flavonoids (Ferric Chloride Test)
About 0.5 g of each extract was boiled with distilled water and filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were added. Green-blue or violet coloration indicated the presence of phenolic hydroxyl group.

Test for saponins
One gram of each extract was boiled with 5.0 ml of distilled water and filtered. To this filtrate about 3.0 ml of distilled water was added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as evidence for the presence of saponins.

Test for anthraquinones
The each (0.5 g) extract was boiled with 10.0 ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5.0 ml of chloroform. The chloroform layer was pipette into another test tube, and 1.0 ml of dilute ammonia was added. The resulting solution was observed for color changes.

Test for terpenoids (Salkowski Test)
To 0.5 g of extract, 2.0 ml of chloroform was added. Then concentrated H_2SO_4 (3.0 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for cardiac glycosides (Keller-Killiani Test)
The extract (0.5 g) was diluted to 5.0 ml in water and than 2.0 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underplayed with 1.0 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar, characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Statistical analysis
All the experiments were conducted in triplicates, and standard deviation was calculated.

RESULTS AND DISCUSSION

Antimicrobial activity
The antimicrobial activity of all the four extracts of plant mixture showed different zones of inhibition with all the tested organisms (Table 1). All the extracts (ethanol, methanol, benzene, ethyl acetate) inhibited the growth of almost all the tested pathogenic bacteria and fungi. Among all the four extracts of mixture, ethanol extracts exhibited strong inhibitory effect against *S. aureus*, *L. acidophilus* and *B. cereus* with maximum zone of inhibition, i.e., 26.0±1.41, 25.5±0.70 and 24.0±1.41 mm, respectively which was comparable or even better than synthetic antibiotics like ampicillin and ciprofloxacin against same tested bacterial strains as shown in Table 1 (Fig. 1a, b, and d). This investigation agreed with the study carried out by Ajaybhan *et al.* [21] in which antimicrobial activity of six medicinal plants against dental pathogens was evaluated. The methanolic extract of *T. chebula* showed maximum zone of inhibition against *S. aureus* (27 mm) and *C. albicans* (26 mm).

Benzene and ethyl acetate extract showed moderate activity against all the tested pathogenic bacteria but highly effective against fungi. Benzene extract recorded highest zone of inhibition against *A. niger* (26 mm) and *A. terrus* (20.5 mm) while ethyl acetate extract was highly effective against *A. flavus* (17.5 mm) and *A. terreus* (20.5 mm) as shown in (Fig. 1c, e, and f). The antibiotic drug fluconazole showed no activity against these fungal strains, so benzene and ethyl acetate extracts can be used as potent herbal antifungal agents against these strains without any risk of side effects. In another study, similar results were observed [22-25] where *Escherichia coli* and *S. typhi* were found to be most susceptible toward antibacterial action of ethyl acetate and benzene extracts In a similar study *in vitro* antimicrobial and antitumor activities of leaf extracts of *Stevia rebaudiana* (Asteraceae) was carried out, and it was found that ethyl acetate extract had effective antibacterial potential against *S. aureus*, *S. typhi*, *E. coli*, *Bacillus subtilis*, *A. hydrophila* and *Vibrio cholera* and antifungal activity against *C. albicans* [26,27].

Antioxidant activity
DPPH radical scavenging activity
The free radical scavenging capacity of extract was determined *in vitro* against common free radical (DPPH). The effect of the antioxidants on DPPH is thought to be due to their hydrogen donating ability [28]. The results indicated that ethanol extract of the mixture at concentration

Table 1: *In vitro* antimicrobial activity (zone of inhibition in mm) of various extracts of the plants mixture against pathogenic bacteria and fungi by agar well diffusion assay

Organism	Ethanol	Methanol	Benzene	Etyl acetate	Ampicillin	Ciprofloxacin	Fluconazole
B. cereus	24.0±1.41	23.5±0.71	11.5±0.70	15.0±0.00	-	25.0±2.83	*
Shigella	22.5±0.70	22.5±0.71	10.0±0.00	15.5±0.71	<10	26.5±0.71	*
A. hydrophila	21.0±0.00	22.0±0.00	10.0±0.00	13.0±0.71	-	25.5±0.71	*
L. acidophilus	25.5±0.70	26.5±1.41	11.0±0.00	12.5±0.71	<10	24.0±0.71	*
S. aureus	26.0±1.41	23.5±0.71	11.5±0.71	14.5±0.71	17.5±0.71	24.5±0.71	*
P. fluorescens	21.5±0.70	21.0±0.00	10.5±0.71	14.5±0.71	-	31.0±4.24	*
S. typhi (A)	14.5±0.70	14.5±0.70	11.5±0.72	13.5±0.71	13.0±1.41	26.0±0.00	*
S. typhi	14.5±0.70	14.5±0.70	12.5±0.72	15.5±0.71	13.0±1.41	26.0±0.00	*
S. typhi (N)	14.5±0.71	15.5±0.72	13.5±0.71	10.0±0.71	11.5±0.71	25.5±0.71	*
S. typhi (S)	15.5±0.72	15.5±0.72	10.0±0.00	10.0±0.00	-	26.0±0.00	*
A. flavus	-	-	17.5±0.71	17.5±0.70	*	*	-
A. niger	-	-	26.0±1.41	15.0±0.71	*	*	-
A. terreus	-	-	20.5±0.71	20.5±0.71	*	*	-
C. albicans	10.5±0.71	10.5±0.71	11.0±0.00	11.0±0.00	*	*	-

*Not tested, -: No activity, *B. cereus: Bacillus cereus, A. hydrophila: Aeromonas hydrophila, L. acidophilus: Lactobacillus acidophilus, S. aureus: Staphylococcus aureus, P. fluorescens: Pseudomonas fluorescens, S. typhi: Salmonella typhi, S. typhi: Salmonella typhi, A. flavus: Aspergillus flavus, A. niger: Aspergillus niger, A. terreus: Aspergillus terreus, C. albicans: Candida albicans*

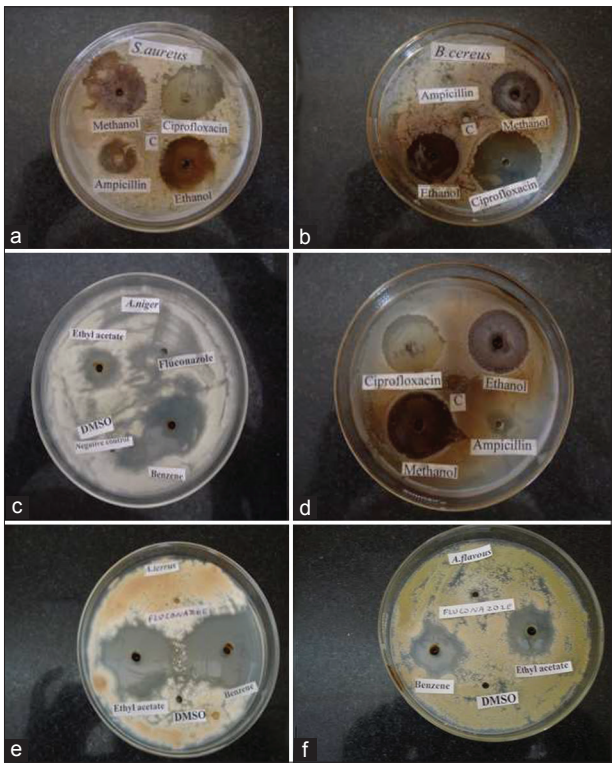


Fig. 1: Inhibitory effect of different extracts of the mixture on pathogenic strains. (a) *Staphylococcus aureus*, (b) *Bacillus cereus*, (c) *Aspergillus niger*, (d) *Lactobacillus acidophilus*, (e) *Aspergillus terreus*, (f) *Aspergillus flavus* by agar well plate method

of 100 µg/ml exhibited maximum inhibition, i.e. 74.54% whereas the inhibition produced by L-ascorbic acid (standard) was 82.77% (Fig. 2). Furthermore, the ethanol extract at other concentrations i.e. 10, 20, 30, 40, 50, 60, 70, 80, 90 µg/ml showed marked DPPH scavenging activity in terms of 35.37, 39.29, 43.66, 49.77, 54.26, 57.49, 62.56, 67.51, 71.08% inhibition, respectively (Fig 2). The amount of ethanol extract needed for 50% inhibition of DPPH radical was 42.55 µg/ml which is higher than IC_{50} value of ascorbic acid, i.e. 43.94 µg/ml (Table 2). Methanol, benzene and ethyl acetate extracts showed 50% reduction of DPPH at concentration of 48.28, 72.11, 83.33 µg/ml respectively. Hence it may be concluded that ethanol extract is approximately as effective as ascorbic acid as an antioxidant.

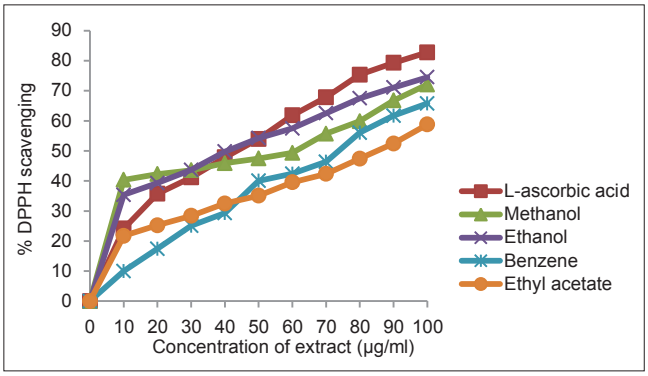


Fig. 2: Scavenging of the 1,1-diphenyl-2-picrylhydazylradical by different extracts of plants mixture

These findings are in conformation with results [29] in which ethanolic extract of *Phellinus linteus* showed the strongest antioxidant activity with IC_{50} value of 17.73±0.27 µg/mL.

Wang *et al.* [15] carried out study on antioxidant activity of water and ethanol extracts of 10 Chinese herbs and discovered that at a concentration of 1.2 mg/ml, *Chrysanthemum indicum* obtained the highest percent scavenging activity (90.03%) among all herbs in ethanol extract and 85.24% in water extract.

Chelating effect on ferrous ions

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agent, the complex formation is disrupted, resulting in a decrease of red colored complex. Measurement of color reduction is the measurement of metal chelating activity. In this assay, various extracts of mixture along with standard compound interfere with the formation of ferrous and ferrozine complex suggesting that they have chelating activity and are able to capture ferrous ions before the formation of ferrozine. The chelating effect of standard and various other extracts of mixture are shown in Fig. 3. It was observed that methanol extract of the mixture showed more chelating activity (76.82%) than did the ethyl acetate extract (72.91%), ethanol extract (62.11%) or benzene extract (50.66%) at concentration of 200 µg/ml (Fig. 3). The IC_{50} value of methanol extract was 103.97 µg/ml which was quite less than the standard (Fig. 3). These results are supported by findings given in the references [8,30-33].

TPC

The TPCs, expressed as GAE with standard curve equation y=0.001x, R^2=0.997 was recorded to be highest in methanol extract (22.2 µg/ml)

Table 2: IC_{50} value of different extracts of the plants mixture in antioxidant systems

Assay	Solvent extract	IC_{50} value (µg/ml)
DPPH radical scavenging assay	L-ascorbic acid (control)	43.94
	Methanol	48.28
	Ethanol	42.55
	Benzene	72.11
	Ethyl acetate	83.33
Chelating effect on ferrous ions	EDTA (control)	18.74
	Methanol	103.97
	Ethanol	145.43
	Benzene	187.78
	Ethyl acetate	86.18

DPPH: 1,1-diphenyl-2-picrylhydazyl, EDTA: Ethylenediaminetetraacetic acid

Table 3: TPCs of extracts of plants mixture

Extracts	TPC (mg/g) as GAE
Mixture	
Ethyl acetate	18.65
Methanol	22.12
Benzene	18.12
Ethanol	20.94

TPC: Total phenolic content, GAE: Gallic acid equivalents

Table 4: Preliminary phytochemical analysis of mixture extracts

Phytochemicals	Test	Inference
Flavanoids	Ferric chloride	+
Alkaloids	Wager's test	+
Free reducing sugar	Fehling test	+
Saponins	Frothing test	–
Tanins	Ferric chloride reagent test	+
Proteins	Xanthoproteic test	+
Cardiac gylcosides	Keller–Killiani test	+
Terpenoids	Salkowski's test	+
Sterols	Salkowski reaction	+
Anthraquinones	Borntrager's test	–

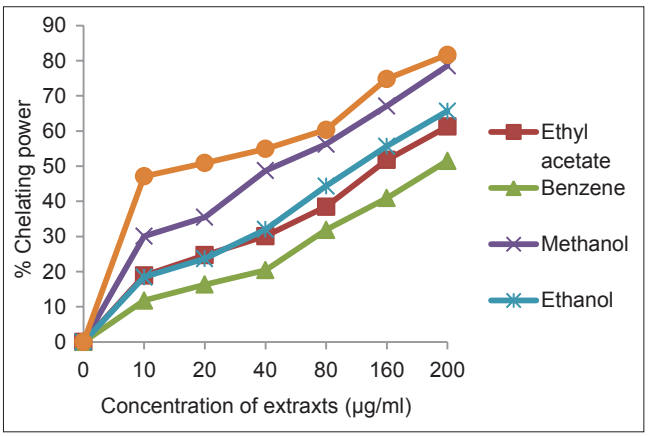


Fig.3: Chelating effect on ferrous ions by different extracts of plants mixture

among the four extracts studied whereas the lowest was recorded in case of benzene extract (18.12 µg/ml) as shown in Table 3.

Phytochemical screening

The phytochemical analysis of the mixture revealed the presence of alkaloids, flavonoids, reducing sugars, cardiac glycosides, tannins, terpinoids and sterols (Table 4). The results obtained from this study revealed that plants contained many bioactive agents. These results are in parallel to the earlier studies conducted on terpens, alkaloids, flavonoids and tannins in *Phyllanthus* species [34-36]. The results of phytochemical test on *Borbo ferruginea* are in conformity with results of the study [37] in which it was reported the presence of alkaloids, tannins, saponins and flavonoids.

CONCLUSION

The extracts of the plants mixture showed significant antimicrobial and antioxidant activity and provide a justification for the therapeutic use of the plants mixture. The present data were suggest that the extracts of the plant mixture could be a potential source of natural antimicrobial and antioxidant that may be of valuable importance for the treatment of diseases. Further studies need to identify unknown phenolic components to establish their pharmacological properties using appropriate assays model.

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