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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SELECTED MEDICINAL PLANTS

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ABSTRACT

The aim of the study was to evaluate the *in vitro* antioxidant and antibacterial activity of five medicinal plants viz., *Acoruscalamus, Pelargoniumgraveolens, Cymbopogonmartini, Cymbopogonnardus* and*Cymbopogon citrates*. Antioxidant activity of the plant was assayed by 1,1-dephenyl-2-picryl-hydrazyl (DPPH). The highest antioxidant activity (68.89±0.31) was observed in*P.graveolens*and the least (14.53±0.08) in *A.calamus*at the lowest concentration *i.e.* 50 µg/ml.However the higher concentrationof plant extractsi.e. 250µg/mldisplayed theeconomically affective dose.*P.graveolens* was observed to be the richest source of phenolic compounds (22.27±0.284mg GAE ml^{-1})whileminimum concentration (7.63±0.046mg GAE ml^{-1}) was measured in *A.calamus*. The ethanolic extracts of five medicinal plants showed inhibitory activity against all the four bacteria in which the diameter of zone of inhibition varied from 17 mm to 24 mm, 20 mm to 26 mm,21mm to 24mm, 18 mm to 24 mm and 16 mm to 22 mm respectively in*A.calamus,C.nardus,P.graveolens,C.citratus,C. martini*.Minimum inhibitory concentration (MIC) assayrevealed that*C.citratus*has highest antimicrobial activity at a minimum concentration (5 mg/ml) against *S. aureus* and *E. coli*.

KEY WORDS: Antioxidant activity, Antibacterial activity, *Escherichia coli*, Minimum inhibitory concentration, *Pelargoniumgraveolens.*

INTRODUCTION

Plants produce a diverse range of bioactive molecules,making them rich source of different types of medicines.Most of the drugs today are obtained from naturalsources or semi synthetic derivatives of natural productsand used in the traditional systems of medicine. Thus it isa logical approach in drug discovery to screen traditionalnatural products. Extracts of many plants are highly efficient against parasitic as well as microbial infections. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time to other for medicinal purposes[1]. The use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. This system of medicine caters to the needs of nearly 70% of the population residing in villages. Besides the demands made by these systems as their raw material, the demands for medicinal plants made by the modern pharmaceutical industries has also increased manifold[2,3].

Many low molecular mass proteins or peptides with antibacterial or antifungal activity have been isolated in recent years from various plants[4,5], and are believed to be involved in a defense mechanism against fungi. Most of these gene-encoded peptides are mobilized shortly after microbial infection to neutralize a broad range of microbes[6]. Many of the antibiotics and other synthetic drugs show sensitization reactions and other undesirable side effects, and there is a feeling that the herbal drugs are relatively safer than others of multifarious nature. Microorganisms develop resistance to the synthetic drugs, antifungal and antibiotics. Another discrepancy of the synthetic drugs and antibiotics is that they may also make interactions with the body system to disturb the metabolic processes. Scientists therefore, are working on the extraction of anti-inflectional compounds including antifungal peptides/proteins from natural sources like plants andanimals. The anti-infectional compounds show broad spectrum bioactivity against infection-causing agents such as bacteria, fungi, protocists, protozoans, viruses, yeastsetc[7].The evaluation of the antioxidant activities of polyphenols from ethno medicinal plants may also benecessary because they are among desired medicinal properties of plants due to their nutraceutical effects[8]. Antioxidants are compounds that can delayor inhibit the oxidation of lipids or other molecules byinhibiting the initiation or propagation of oxidizing chainreactions. The antioxidant activity of polyphenols ismainly due to their redox properties, which can play animportant role in adsorbing and neutralizing free radicals,quenching oxygen, or decomposing peroxides.Antioxidant activities of polyphenols

have beensuggested to exert beneficial pharmacological effects onneurological disorders on the basis of *in vitro*observations[9,10]. Polyphenolic compounds in plant, including thecatechins, exert anticarcinogenic, antimutagenic andcardioprotective effects linked to their free radicalscavenging[11]. They are reported to bechemopreventive agents by lowering cholesterol androughly limit cell damage[12]. Inaddition to their individual effects, antioxidants interact insynergistic ways and have sparing effect in which onemay protect another against oxidative destruction. Thesejustify the overwhelming interest in finding naturallyoccurring antioxidants for use in foods or medicinalmaterials to replace synthetic antioxidants[10].Thus, our present study is focussed on five medicinal plants namely *Acoruscalamus*L, *Pelargonium graveolens*L, *Cymbopogon martini* L, *Cymbopogonnardus*L *and Cymbopogoncitratus* L to determine their antioxidantand antimicrobial activity.

MATERIALS AND METHODS

Collection of medicinal plants

The five medicinal plants for study were collected from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India and were grown in the green house of the College of Forestry, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad U.P. The plant materials for analysis were cleaned and powdered. The botanical names, family names, English names and parts used are presented in Table 1.

Extraction of Plant Materials

The fresh leaves of plants (Table 1) were washed thoroughly with sterile distilled water and air-dried in the shed at room temperature for two weeks, after that it was grinded to a uniform powder. Methanol extracts were prepared by soaking 10 g each of the dry powdered plant material in 100 ml ethanol at room temperature for 48 hr. The extracts were filtered through cotton wool. The extracts were concentrated using a rotary evaporator with hot water bath set at 40^{o}C. The percentage yield of extracts ranged from 5-20% (w/w).

Collection of Microorganisms

The bacterial strains used in this study were *Escherichia coli, Bacillussubtilis, Staphylococcusaureus*and *Vibrio cholerae.* All of them were obtained from the microbial culture collection bank of the Department of Microbiology and Fermentation Technology, SHIATS, Allahabad. Bacterial cultures were maintained on nutrients agar slants in the Biochemistry Laboratories, subcultured and stored at 4^{o}C. The strains were inoculated in the Nutrient broth (pH 7.0) and incubated at 37^{o}C for 24 hrs.

Estimation of Antioxidant activity

The antioxidant activity of the plant extracts was examined on thebasis of the scavenging effect on the stable DPPH free radical activity[13].Ethanolic solution of DPPH (0.05 mM)(300 µl) was added to 40 µl of extract solution with different concentrations(0.02 - 2 mg/ml). DPPH solution was freshly prepared andkept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and themixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517nm. Ethanol was used to set the absorbance zero. A blank samplecontaining the same amount of ethanol and DPPH was alsoprepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed aspercentage of inhibition were calculated according to the followingequation. Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100

Where, AAand AB are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample requiredfor 50% inhibition was determined and represented as IC_{50}value for each of the test solutions.

Estimation of Total Phenol content

Estimation of TPC was done by the method of Ragazzi and Veronese[14].Weighed 10 mg plant extract was dissolved in 10 ml of 50% CH_3OH : H_2O (1:1), kept overnight at room temperature and in its 1.0 ml, 1.0 ml of Folin's Reagent (1N) and 2.0 ml of Na_2CO_3 (20%) were added subsequently. The test mixture was mixed properly on cyclomixer, left at room temperature for 30 min and volume maintained up to 25 ml with distilled water. The absorbance of test mixture was read at λ_{max} 725 nm on Spectrophotometer. Gallic acid was used as standard.

Antimicrobial Activity Test

The antimicrobial test was carried out by agar well diffusion method.

Agar well diffusion method

The antibacterial activity of five crude ethanolic extracts of medicinal plants against four bacteria was evaluated by using agar well diffusion method[15]. Microbes were inoculated with 100µl of standardized inoculums (1.5×10^8 CFU/ml)of each selected bacterium(in triplicates) and spread with sterile swabs, wells of 8 mm size were made with sterile borer

into agar plates containing the bacterial inoculums and the lower portion was sealed with a little molten agar medium. 100µl volume of the plate extract was poured into a wall of inoculated plate. Chemical preservative, acetic acid was used as a positive control which was introduced into a well inside of plant extract. Solvent, ethanol was used as negative control which was introduced into a well inside of plant extract. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar[16]. After incubation for 24 hrs at 37^0C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters.

Determination of minimum inhibitory concentration (MIC) of ethanolic medicinal plant extract against gram positive and gram negative bacteria

The MIC is defined as the lowest concentration of the antimicrobial agents that will inhibit the visible growth of microorganisms.

Macro dilution broth method

In the macro dilution broth method, a two-fold serial dilution of the plant ethanolic extracts were prepared in sterile Mueller-Hinton broth to achieve a decreasing concentration ranging from 160 to 1.25 mg/ml in eight sterile test tubes labeled 1 to 8. Each dilution was seeded with 100µl of the standardized bacterial inoculums (1.5×10^8 CFU/ml). The inoculated culture tubes were incubated at 37^0C for 18 to 24 hrs. A set of tubes containing only seeded broth (i.e. without plant extract) were kept as control. The lower concentration that did not permit any visible growth when compared with the control was considered as the MIC.

RESULTS

Results obtained in the present study revealed that the tested five medicinal plants extracts posses potential antioxidant activityand antimicrobial activity against *Escherichia coli*, *Bacillussubtilis*, *Staphylococcusaureus*and *Vibrio cholerae.* DPPHstable free radical method is a sensitive way to determinethe antioxidant activity of plant extracts[17,18].Table 2 showed the amount ofeach extract required for 50% inhibition of DPPH activity(IC_{50}). IC_{50} values are inversely related to the antioxidant activity. Ethanolic extract of *Pelargoniumgraveolens* (Table 2) hasstrongest antioxidants scavenging property anddisplayed the percentage of activity (68.89%) while lowest scavenging activity was seen in *Acoruscalamus* (14.53%) at 50 µg/ml concentrations. However just higher concentration of all plant extracts*i.e.*250 µg/ml was perceived to be potent and economically affective dose as antioxidant than the other

higher concentrations of extracts. The free radical scavenging activities were evaluated by using BHT as standard. The IC_{50} value was seen much higher (220µg/ml) in *Acoruscalamus* and lowest in *Pelargoniumgraveolens* (Table 3).

Pelargonium graveolens(22.27±0.284 mg GAE ml^{-1}) displayed the higher content of total phenol while*Acoruscalamus*(7.63±0.046 mg GAE ml^{-1}) showed the minimum. *Cymbopogon martini* (10.01±0.098 mg GAE ml^{-1}) had the bit higher content of total phenol than*Cymbopogonnardus*(9.1±0.095 mg GAE ml^{-1}). *Cymbopogon citrates* (15.73±0.112 mg GAE ml^{-1}) recorded 1.5 times higher content than *Cymbopogon martini* and 1.5 times lower than *Pelargonium graveolens* (Table 3).

The leaf extract of *Acoruscalamus*showedthe highest antibacterial activity of *E. coli* with 24 mm zone of inhibition(figure 5)and least activity recorded in *B. subtilis* (17 mm) (figure 3).*S. aureus* (22 mm) expressed the higher antibacterial activity(figure 4)as compared to *V. cholerae* (18 mm) (figure 6).Ethanolic leaf extract of *Cymbopogonnardus* showed highest antibacterial activity of 26 mm against *S. aureus* and least activity measured in *V. cholerae* (20 mm). *B. subtilis*showed(23 mm) little bit higher activity as compared to *E. coli* (22).*Pelargonium graveolens* had maximum activity of 24 mm in *V. cholerae*. *B. subtilis* and *E. coli* showed the similar activity and *S. aureous*measured a bit lower activity as compared to both of these bacteria. Extracts of*Cymbopogoncitrates* exhibit highest activity against *E. coli* and *V. cholerae* (22 mm) and lowest against *S. aureus* (18 mm). *B. subtilis* (21 mm) measured little bit lower activity than *E. coli* and *V. cholerae*. *Cymbopogon martini* leaf extract showed almost similar zone

Minimum inhibitory Concentration (MIC) of ethanolic leaf extract of five medicinal plants against bacteriais shown in Table 5.The leaf extract of *Acoruscalamus*in ethanol showed MIC of 20 mg/ml against *B. subtilis* and *E. coli* whereas 10 mg/ml and 5 mg/ml against *V. cholerae*and *S. aureus* respectively. *Pelargonium graveolens*ethanolic leaf extract displayed MIC 20 mg/ml against *S. aureus* and 10 mg/ml against *B. subtilis* and *V. cholerae* whereas 5 mg/mlMICwas noted against *E. coli*.*Cymbopogon martini* extracts gave MIC 20 mg/ml against *B. subtilis* and *E. coli*while MIC 10 mg/ml and 5 mg/ml demonstrated against *S. aureus* and *V. cholerae* respectively. *Cymbopogonnardus* extract measured MIC 20 mg/ml against *S. aureus* and *V. cholera* whereas MIC 10 mg/ml and 5 mg/ml was noted against *E. coli* and *B. subtilis* respectively.Ethanolic leaves extract of *Cymbopogoncitrates* showed MIC 5 mg/ml against *S. aureus* and *E. coli*. Even as MIC 10 mg/ml and 20 mg/ml reported against

V. cholerae and *B. subtilis* respectively. Plant extract of *Acoruscalamus* and *Cymbopogoncitratus* showed MIC 5 mg/ml against *S. aureus* while 20 mg/ml MIC showed against *B. subtilis*in extracts of *Acoruscalamus*, *Cymbopogon martini* and *Cymbopogoncitratus*. Whereas MIC 10 mg/ml was found against*V. cholera* when ethanolic extract of *Acoruscalamus*, *Pelargonium graveolens* and *Cymbopogoncitratus* were used.

DISCUSSION

It has been shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples and standard to a certain extent[19] and hence are said to be strongly dependent on the extract concentration. The DPPH radical has been used widely to test the potential of the compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron[20]. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit the lipid oxidation[21]. A strong correlation has been observed between the phenols and antioxidant activity. Also strong relationship between total phenolic content and antioxidantactivity has been reported byJavanmardiet al.[22]. In the present study ethanolic extract of *Pelargonium graveolens*displayed highest percentage of free radical scavenging activity (68.89%) at 50 µg/ml concentrations. Total phenolicscontent are plant metabolites characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions[23]. Besides, the phenolic compounds possess multiple biological properties such as antitumor, antimutagenic and antibacterial properties, and these activities might be related to their antioxidant activity[24]. Present study showed the significant relationship between antioxidant activity and total phenolics content. *Pelargonium graveolens*had the highest antioxidant activity and total phenolic content. *Acoruscalamus*was noted to be the lowest phenolic content so it had the lowest antioxidant activity.

Plants are important source of potentially usefulstructures for the development of new chemotherapeuticagents. The first step towards this goal is the *in vitro*antibacterial activity assay[25]. Many reports areavailable on the antiviral, antibacterial, antifungal,anthelmintic, antimolluscal and anti-inflammatoryproperties of plants[26,27,28]. Some of theseobservations have helped in identifying the activeprinciple responsible for such activities and in thedeveloping drugs for the therapeutic use in humanbeings.However, not many reports are

available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection.

In the present study, the ethanol leaves extracts of *Acoruscalamus,Pelargonium graveolens,Cymbopogon martini, Cymbopogonnardus and Cymbopogoncitratus*displayed the activity against *B. subtilis, E. coli, V.cholerae* andS. *aureus,* and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The antibacterial activity of*A.calamus* rhizome and leaf extracts obtained with different solvent *viz.* petroleum ether and chloroform has earlier been reported against *S.aureus, E.coli*and *B.subtilis*[29]. All studied medicinal plants extract except *Cymbopogonnardus*exhibited significantly inhibitory activity againstS. *Aureus*while all of themhave near about the same inhibitory activity against *E. coli*[30]. The minimum inhibitory concentration (MIC) of ethanolic plant extracts against the bacterial strains varied.Shariffet al.[31] suggested that the MIC of 2.0 and 4.0 mg/ml was found against all thetested bacteria like *Bacillus subtilis, Escherichia coli,* S. *aureus,V.cholera,Xanthomonasaxonopodis*pv. *malvacearum, Xanthomonasvesicatoria*when absolute alcohol and chloroform extracts of *P. minima* leaf were used.In the present study ethanolic extract of *Cymbopogoncitratus*showed 5mg/ml MIC against S. *aureus* and *E. coli* whereas 10 mg/ml against *V. cholerae.*

Table 1.Medicinal Plants used in the study.

Botanical Name	English Name	Part used	Family	Medicinal use
Acoruscalamus	Bach	Leaf	Araceae	Brain tonic, Fever,Asthma digestive problems
Pelargoniumgraveolens	Geranium	Leaf	Geraniaceae	Cough, Kidney pain, gastrointestinal ailments.
Cymbopogon martini	Palmarosa	Leaf	Poaceae	Skin disease, treatment of baldness.
Cymbopogonnardus	Citronella	Leaf	Poaceae	Muscular pain, Nervous disorder.
Cymbopogoncitratus	Lemongrass	Leaf	Poaceae	Insect repellent, digestive problems.

Table 2. Percentage DPPH Scavenging Activity in leaves of medicinal plants.

Conc. (µg/ml)	*Acoruscalamus*	*Cymbopogonnardus*	*Cymbopogon martini*	*Cymbopogoncitratus*	*Pelargonium graveolens*
50	14.53±0.08	51.78±0.21	60.61±0.25	65.38±0.03	68.89±0.31
250	56.55±0.17	75.78±0.215	75.84±0.33	85.56±0.13	84.54±0.04
500	66.73±1.66	75.69±0.24	80.93±0.08	86.64±0.05	84.89±0.16
750	78.86±0.03	79.29±0.06	82.28±0.03	85.55±0.27	82.76±0.28
1000	82.03±0.07	84.17±0.22	84.69±0.13	88.08±0.49	87.49±0.18
S.E	1.092	0.278	0.323	0.229	0.351
C.D	2.295	0.584	0.679	0.481	0.738

* Data represented as mean ± S.D. from three individual experiments.

Table 3.IC_{50} value in ethanolic extracts of medicinal plants leaves.

Plants	IC_{50}(µg/ml)	Total penol content (mg GAE ml^{-1})
Acoruscalamus	220	7.63±0.046
Cymbopogonnardus	50	9.1±0.095
Cymbopogon martini	40	10.01±0.098
Cymbopogoncitratus	40	15.73±0.112
Pelargoniumgraveolens	38	22.27±0.284

Table 4. Antimicrobial activity of medicinal plants extract against bacteria.

Ethanolic plants extract	Determination of inhibitation zone (mm)[a]			
	B. subtilis	***S. aureus***	***E. coli***	***V. cholera***
Acoruscalamus	17±0.33	22±0.94	24±0.47	18±0.47
Cymbopogonnardus	23±1.24	26±0.47	22±0.94	20±0.81
Pelargoniumgraveolens	22±0.47	21±0.47	22±0.94	24±0.47
Cymbopogoncitratus	21±0.81	18±0.94	22±0.94	22±0.94
Cymbopogon martini	21±0.47	17±0.47	22±0.94	16±0.47
Acetic acid (positive control)	22±0.47	17±0.47	22±0.47	25±0.47
Ethanol (negative control)	-	-	-	-

-No activity; [a] –values, including diameter of well (5 mm), are means of the three replicates;± standard deviation

Table 5.Minimum inhibitory Concentration (MIC) of ethanolic leaves extract of medicinal plants against bacteria.

Plants	MIC (mg/ml)			
	B. subtilis	***S. aureus***	***E. coli***	***V. cholera***
Acoruscalamus	20	5	20	10
Pelargonium graveolens	10	20	5	10
Cymbopogon martini	20	10	20	5
Cymbopogonnardus	5	20	10	20
Cymbopogoncitratus	20	5	5	10

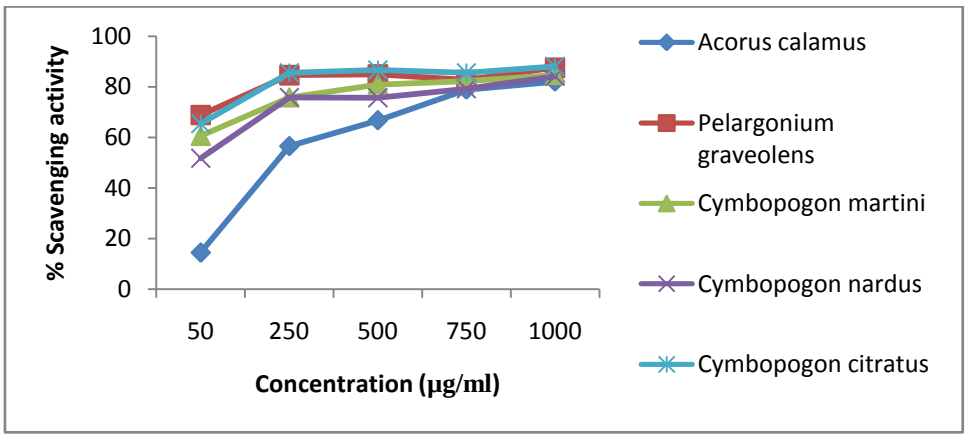

Fig1. Percentage DPPH scavenging activity in leaves of medicinal plants.

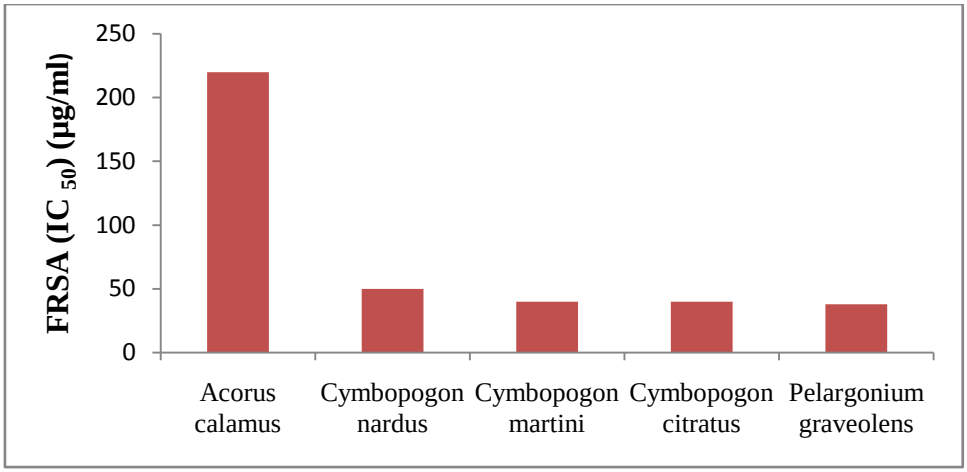

Fig 2. IC_{50} value in ethanolic extract of medicinal plants leaves.

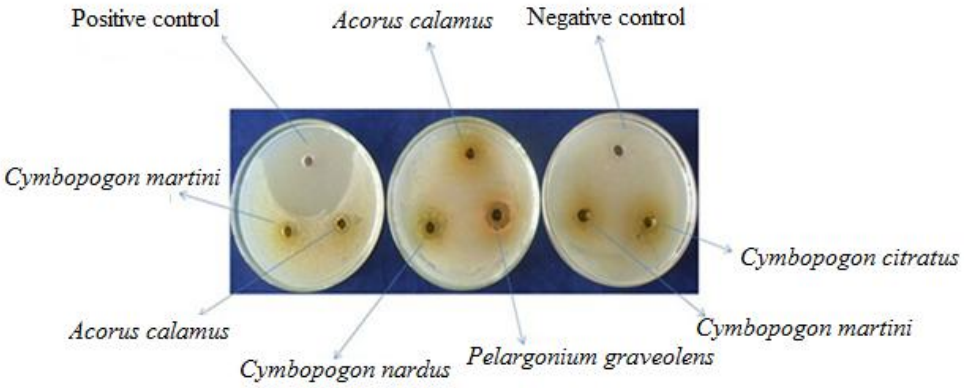

Fig 3.Antibacterial activity of ethanolic extracts against *B. subtilis*.

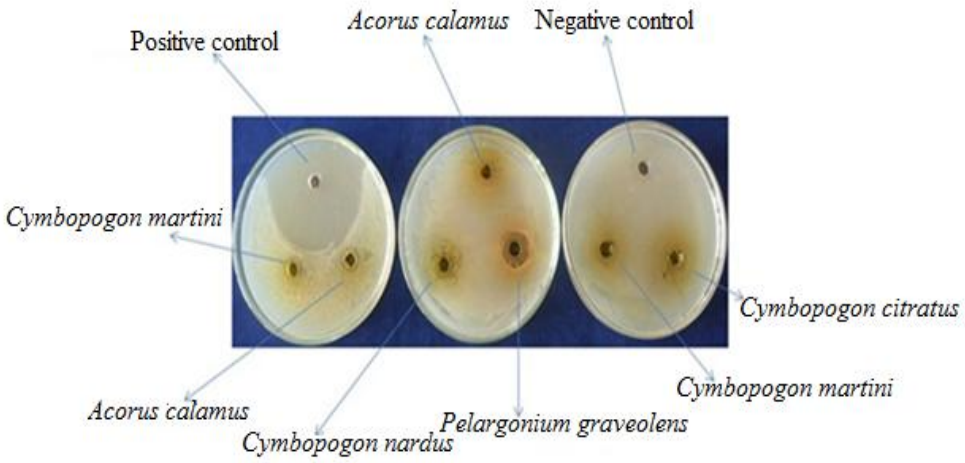

Fig 4.Antibacterial activity of ethanolic extracts against *S. aureus*.

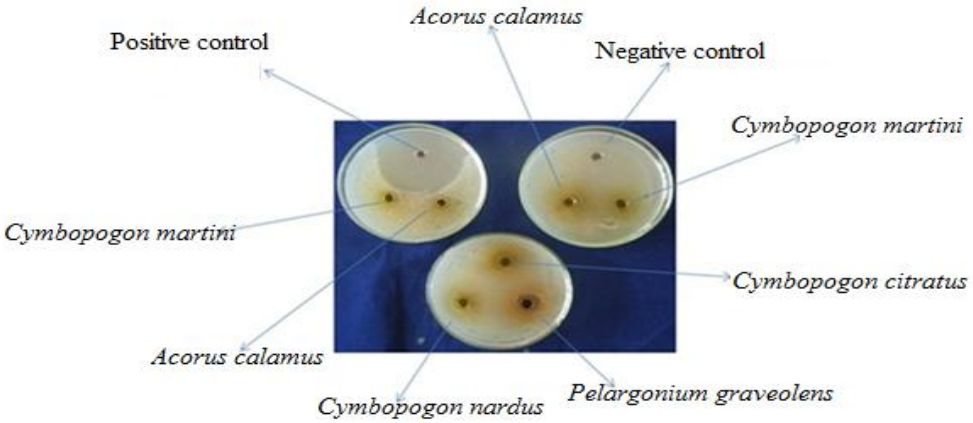

Fig 5.Antibacterial activity of ethanolic extracts against *E. coli*.

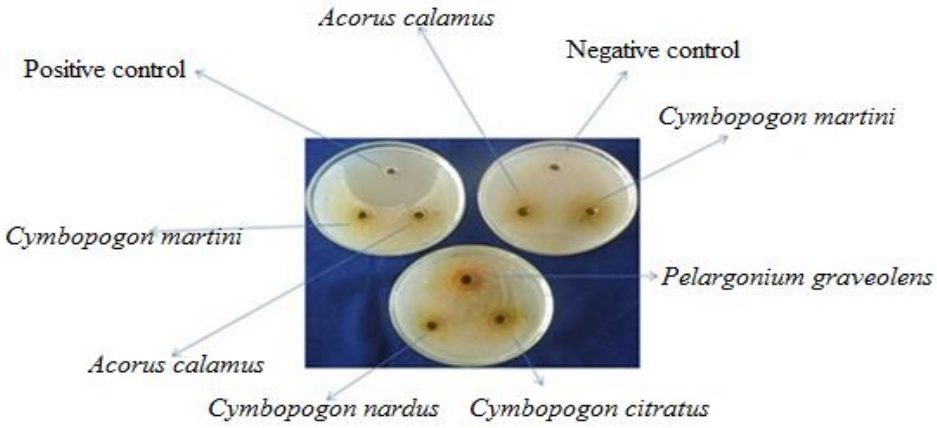

Fig 6.Antibacterial activity of ethanolic extracts against *V. cholerae*.

CONCLUSION

The antioxidant activity and total phenolic contents of selected medicinal plants wereevaluated, and the potential antioxidant activity of plants was assessed for the first time.Positive correlations between antioxidant activity and total phenolic content suggest that theantioxidant activities of the medicinal plants can be mainly ascribed to their phenol compounds. Further research isnecessary to determine the identity of the antibacterialcompounds from within these plants and also to determinetheir full spectrum of efficacy. However the presentstudy of *in vitro* antibacterial evaluation of some plantsforms a primary platform for further phytochemical andpharmacological studies to discover new antibiotic drugs.

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