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Comparative Study on Phytochemical Constituents, Antimicrobial and Plasmid Curing Property of *Aloe vera* and *Ocimum sanctum*

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Abstract : *This study investigated phytochemical content, antimicrobial and plasmid curing property of four extracts (viz.petroleum ether, ethanol, distilled water and acetone) by reflux extraction technique of leaves of Aloe vera and Ocimum sanctum. Qualitative and quantitative analysis of secondary metabolites were carried out. Agar well diffusion method was used for antibacterial activity against Escherichia coli and Bacillus cereus and plasmid curing property of phytoextracts was carried out. The result revealed that the tested plant materials contained appreciable amounts of total alkaloids (16.00- 71.00 mg/gm leaves), total phenols (10.233- 40.267 µg g^{-1}fw), total saponins (10.267- 65.167 µg g^{-1}fw) zone of inhibition (0.00- 12.33 mm) and plasmid curing property (81- 84%). Generally higher extract yields, secondary metabolites, antimicrobial activity and plasmid curing property were obtained using ethanol as a solvents, as compared to the respective other solvents.*

Keywords: Phytochemicals, Plasmid curing, Reflux extraction, *Aloe vera*, *Ocimum sanctum*, Agar well diffusion, *Escherichia coli* and *Bacillus cereus.*

introduction

Plants are one of the most important sources of medicines. Today the large numbers of drugs in use are derived from plants. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice (Atal and Kapoor, 1989).

Aloe vera belongs to the Liliaceae family, of which there are about 360 species. It is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities. The gel of *A. vera* was used to treat stomach ailments, gastrointestinal problems, skin disease, constipation, radiation injury, inflammatory effect, healing wounds, burns, ulcer and diabetes.

Ocimum sanctum L. (Tulsi) from family Lamiaceae is an erect, much branched sub-shrub 30-60 cm tall. Tulsi is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. It is cultivated for religious and medicinal purposes and for its essential oil. It is also a source of aroma compounds and essential oils containing biologically active constituents that possess insecticidal and nematicidal properties (Deshpande and Tipnis, 1997). However, the antioxidative potential of herbs and spices is well correlated with the presence of phenolic compounds due to its redox properties, which permit them to act as reducing agents, hydrogen donators and singlet oxygen quenchers.

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms

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(endotherms). *Escherichia coli* is associated with the gastrointestinal tract of man and animals. *E. coli*, a facultative anaerobe of wide distribution in the environment, has been implicated in the cause of urinary tract infections, meningitis, sepsis, wound infections, noscomial pneumonia and arthritis.

Bacillus cereus is a Gram-positive, rod-shaped, endospore forming, facultative aerobic bacterium. it has been reported to cause local and systemic infections, as an opportunistic pathogen, especially among immunocompromised patients, newborns, and patients with surgical wounds. *B. cereus* can cause ocular infections such as keratitis, endophthalmitis, and panophthalmitis.

This work was conducted to investigate the qualitative, quantitative, antibacterial and plasmid curing property of phytoextracts.

Materials and Methods

Collection of samples

The medicinal plants used for the experiment were *Aloe vera* and *Ocimum sanctum***.** These plants were collected from the wild areas of Allahabad and identified according to the different flora.

Collection of test organisms

Two bacterial strains used in this study *Escherichia coli* and *Bacillus cereus* the MDR bacterial strains were collected from the "Microbial Culture Collection Bank", Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad.

Preparation of plant extracts

Fresh leaves of *Aloe vera and Ocimum sanctum* were thoroughly washed and chopped into small pieces and dried in an oven at 50°C and grinded into powder; separately refluxed with 50 ml of each solvent (viz distilled water, ethanol, petroleum ether and acetone) continuously for 3 h using the reflux apparatus by maintain its temperature according to the boiling point of each solvent. Thereafter, the resulting extracts were filtered using Whatman No. 1 filter paper and stored in refrigerator for further use in the experiment.

Qualitative analysis of extracts

The phytochemical screening of the crude extracts was carried out in order to ascertain the presence of secondary metabolites such as alkaloids, phenols, flavonoids, steroids, terpenoids, saponins, anthraquinones, glycosides, tannins using standard methods of analyses (Harborne, 1973).

Quantitative analysis of extracts

Determination of total alkaloids

10 ml of each extract was evaporated thereafter it was dissolved in 10 ml of 0.1 N HCl solution. This solution was titrated against 0.1 N NaOH solution using a drop of methyl red as a indicator to determine amount of HCl left after being neutralized by the alkaloids (Dayanand, 1998).

Determination of total phenols

10 ml of each of the extract was evaporated. The residue was dissolved in 5 ml of distilled water. 0.5 ml of Folin-Ciocalteau reagent was added in the solution. After 3 min, 2ml of 20% Na_2CO_3 solution was added to each test tubes then it was mixed thoroughly. All the test tubes were placed in boiling water for exactly 1 min, cooled and absorbance was measured at 650 nm against a reagent blank (Malick and Singh, 1980).

Determination of total saponins

10 ml of extract was evaporated; residue was dissolved in 20 ml of $MgCO_3$ saturated solution and filtered. Then 1 ml of solution was taken in 50 ml flask, it was mixed with 2 ml of 5% $FeCl_3$ solution. It was allowed to stand for 30 min to develop red color and absorbance was recorded at 380 nm against a reagent blank (Brunner, 1984).

Antibacterial activity of extract

The antibacterial activity was assessed by agar well diffusion method. Sterile agar (at 45°C) was poured into sterile Petri dishes, which had been inoculated with the test organisms. The plates were allowed to gel for an hour. Wells (10 mm diameter) were made with the aid of flamed cork borer on the surface of the agar plates. About 1 ml of each of the phytoextracts were evaporated and residue was dissolved in 10 ml of 10% DMSO and 0.1 ml were delivered into each of the wells. These were incubated at 37°C for 24 h. The presence of zones of inhibition was regarded as the presence of antimicrobial action. From the inhibition zones seen, antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured (Agarry *et al.,* 2005).

Plasmid curing property of extracted phytochemicals

The ethanol extracts of these plants (*Aloe vera* and *Ocimum sanctum*) were tested for curing of antimicrobial resistance against multidrug resistance *E. coli* isolates. Small inoculums of bacteria was grown overnight at 37ºC in nutrient broth (i.e. peptone water) containing inhibitory concentration of medical plant extract, giving in complete inhibition, using acridine orange as a control. The cultures were plated on agar and isolated colonies tested for antibiotic resistance. Isolated colonies were then replicated on to nutrient agar and nutrient agar containing antibiotic ampicillin. The colonies which grew on nutrient agar but failed to grow in presence of antibiotic were considered as putative cured derivatives. The percentage curing efficiency was expressed as number of colonies with cured phenotype per 100 colonies tested. The agar plate was used as negative control in plasmid curing experiment (Deshpande *et al.,* 2001).

Results and Discussion

Qualitative analysis of extracts

The phytochemical active compounds of *Aloe vera* and *Ocimum sanctum* were qualitatively analyzed and the results are presented in Table 1 and 2. Based on the presence or absence of colour change indicate positive and negative results.

Quantitative analysis of extracts

The quantitative analysis of the extracts was carried out in order to quantify secondary metabolites such as alkaloids, phenols and saponins in all four extracts as Table 3 that the alkaloids are comparatively higher in *Ocimum* than *Aloe*. Similarly phenols were also quantified and shows higher yield in *Ocimum* lower in *Aloe*. Ethanolic extracts of both the plants shows highest yield than other extracts.

Antibacterial activity of extract

The antibacterial screening of the *Aloe vera* and *Ocimum sanctum* extracts against the chosen organisms are tabulated in Table 4 (Fig. 1 and 2). The inhibitory zone diameter produced, ethanolic extract shows best result. For both microorganisms *Ocimum* have more inhibition zone than *Aloe*.

Plasmid curing property

The crude ethanolic extract of both plant showed plasmid curing activity as shown in Table 5 and depicted in Fig 3. *Ocimum* shows best result with 84% cured colonies than *Aloe*.

The reasons why ethanol extraction gave superior yields may be attributed to the followings: (i) The use of organic solvent that can easily be evaporated than water, (ii) The high temperature used (heating), (iii) Most components of this plant are organic compounds which easily dissolves in an organic solvent. However, percentage yield of the crude extract was observed to be generally low, and might even be smaller when these bioactive agents are to be obtained in their pure form, this situation give an evidence that are at low concentration is very low in the plant. It is clear that ethanol extracts gave higher yield percentages than other extracts in both plant

Table 1 Results of qualitative analysis of the *Aloe vera*.

Phytochemicals	Solvents			
	D.W.	Ethanol	Petroleum ether	Acetone
Alkaloids	+	+++	++	+
Phenols	+	++	+	+
Flavonoids	+	+++	+	++
Steroids	-	+	+	+
Terpenoids	-	+	+	+
Saponins	-	+++	+	++
Anthraquinones	-	-	-	-
Glycosides	-	++	+	+
Tannins	+	+	+	+

Table 2 Results of qualitative analysis of the *Ocimum sanctum.*

Phytochemicals	Solvents			
	D.W.	Ethanol	Petroleum ether	Acetone
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	++	+
Steroids	+	+	+	+
Terpenoids	+	+++	++	-
Saponins	+	+++	++	+
Anthraquinones	-	-	-	-
Glycosides	-	+	-	+
Tannins	+	+	+	+

(Where + shows presence and – shows absence)

Table 3 Total Alkaloids, phenols and saponins of *Aloe vera* and *Ocimum sanctum.*

Tested variables	Alkaloids (mg/gm leaves)				Phenols (µg g^{-1}fw)				Saponins (µg g^{-1}fw)			
Solvents	PE	Ethanol	D.W.	Acetone	P.E	Ethanol	D.W.	Acetone	P.E	Ethanol	D.W.	Acetone
Aloe vera	31.00	61.66	26.00	41.00	17.23	37.23	13.16	10.23	32.26	53.23	10.26	21.167
Ocimum sanctum	27.66	71.00	16.00	36.33	17.26	40.26	35.20	13.46	32.30	65.16	21.26	43.16

Table 4 Zones of inhibition of *Aloe vera* and *Ocimum sanctum* against the *E. coli* and *B. cereus* (mm).

Tested variables	*Aloe vera*					*Ocimum sanctum*				
Solvents	Control	PE	Ethanol	D.W.	Acetone	Control	P.E	Ethanol	D.W.	Acetone
E. coli	0	2.33	14.33	0.00	6.66	0	8.33	10.66	6.33	9.00
B. cereus	0	0.00	11.33	0.00	8.33	0	6.00	12.33	9.33	8.33

Table 5 Plasmid curing property of plant extracts.

Ethanolic phytoextracts	No. of colonies tested	No. of colonies cured
Aloe vera	100	81
Ocimum sanctum	100	84

samples (Raphael, 2012).

In antimicrobial assays, the inhibitory zone diameter produced is the result of the growth of the test organisms and the diffusion of the test agents through the agar, both events occurring simultaneously. There was no activity against *E. coli* and *Bacillus cereus* for both the typed culture in some solvents; this may be due to the absence of some secondary metabolites or the presence of some in low concentration.

In plasmid curing property it has been suggested that for a synergistic effect, the curing percentage in the test plate (plate containing the plant extract and the antibiotics) should be greater than that in the control plate (plate containing extract-free base agar layer) by at least 84% for *Ocimum* and 81% for *Aloe*.

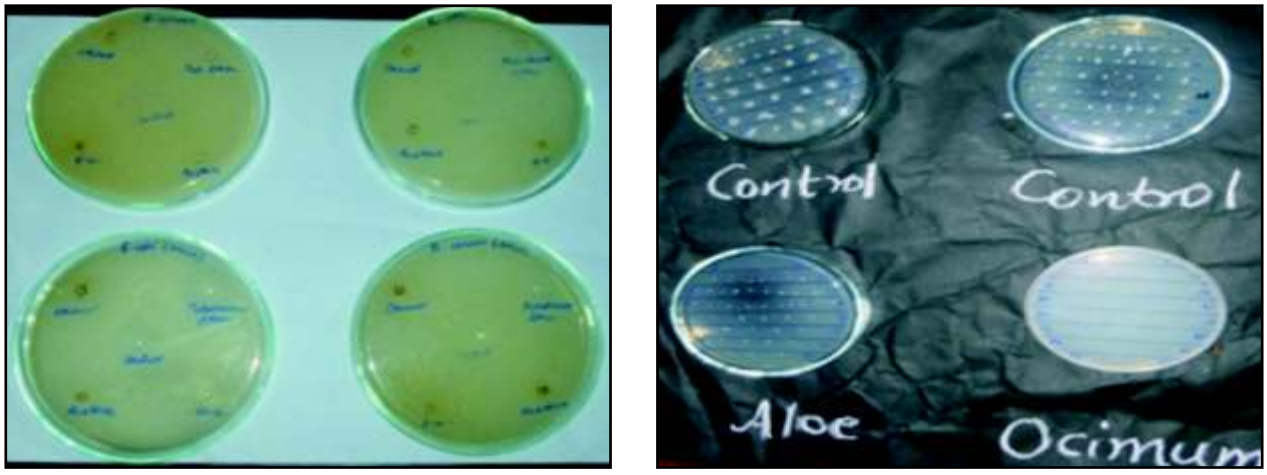

Fig. 1 Agar plates showing zones of inhibition.

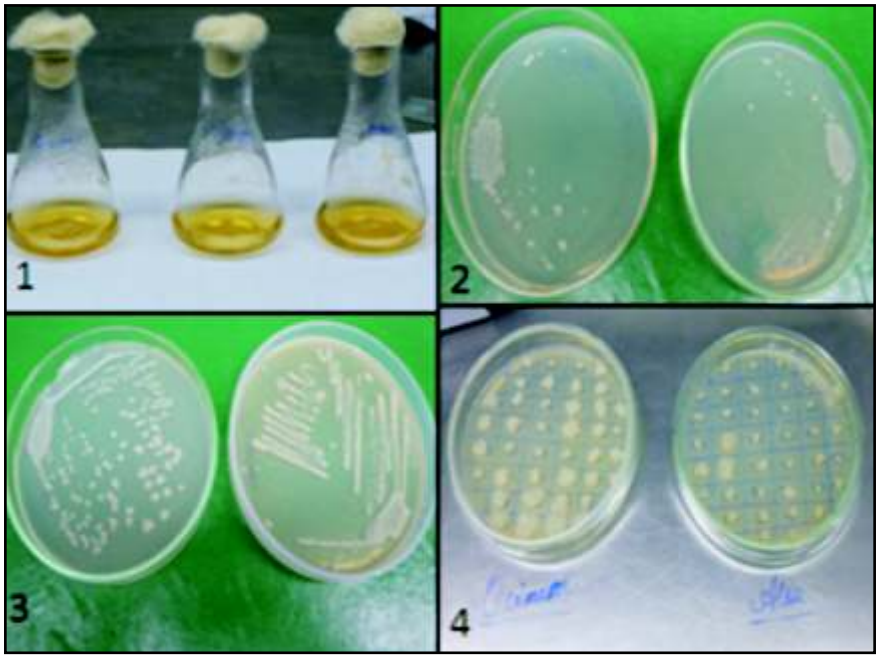

Fig. 2 (1) Nutrient broth having plant extract and bacterial culture; (2) Agar plates having bacterial culture treated with *Aloe;* (3) Agar plates having bacterial culture treated with *Ocimum;* (4) Isolated bacterial colonies.

However it was also observed that for *E. coli* it was only ampicillin that did not show activity against it. This is because of the fact that *E. coli* develops resistance to ampicillin. Conclusively, the result of the interaction of the leaves of the plant with the antibiotics investigated in this study was thought to be basically due to the effects of the phytochemical constituents of the plants (Lee *et al.,* 2004).

The present study suggested that among all solvents used, the ethanolic extract of *Aloe vera* and *Ocimum sanctum* contained more saponins, alkaloids and phenols. Phenols showed comparatively more antibacterial activity and was found to be the best in curing of plasmid.

The result depicted the use of these plants in treating microbial infection and showed that

Aloe vera and *Ocimum sanctum* could be exploited for new potent antimicrobial and plasmid curing agents.

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