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IN-VITRO ASSESSMENT OF ADDITIVE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF EMBLICA OFFICINALIS AND AZADIRACHTA INDICA LEAF EXTRACT COMBINATION

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ABSTRACT

OBJECTIVE: To evaluate the additive antibacterial and antioxidant activity of ethanolic mixture of *Emblica officinalis* and *Azadirachtaindica* leaf extract.

METHOD: The ethanolic extraction of Amla and Neem leaf was carried out by using cold maceration method. The technique of disc diffusion was implemented to evaluate the *in-vitro* antibacterial activity against *E. coli* and *B.subtilis.*The *in-vitro*antioxidant activity of varying concentrations of 20, 40, 60, 80, and 100µg/ml was analyzed using the DPPH free radical scavenging method.

RESULT: The *in-vitro* antibacterial activity of combined leaf extract determined by measuring the zone of inhibition. It shows additive antibacterial activity against the tested organism. The *in-vitro* antioxidant activity shows synergistic effect on ethanolic mixture of leaf extract in a dose dependent manner.

CONCLUSION: The present study concludes that the ethanolic mixture of *E.officinalis* and *A.indica* leaf extractposses additive antibacterial effect and synergistic antioxidant activity.

KEYWORDS:Antibacterial, Antioxidant, Ethanolic extract, Disc diffusion method, DPPH assay.

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INTRODUCTION

Bacterial infections can damage the throat, lungs and skin along with other bodily components. Antibiotics and a variety of synthetic antibacterial medications are Mostly used to treat bacterial disorders. Due to toxicity, side effects, the emergence of resistant strains of organisms, and the buildup of drug residues in bodily tissues and fluids.Phytochemicals and plant extracts with recognized antibacterial activities may play a crucial role in therapeutic procedures[1].

Emblica officinalis and *Azadirachta indica* belongs to the family Euphorbiaceae and Meliaceaerespectively have antioxidant and antibacterial activity due to the presence of alkaloids, tannins and flavanoids [2]. The motive of the current study is to determine whether the combined antioxidant and antibacterial properties of Amla and Neem leaf extracts have an additive impact.

MATERIALS AND METHOD

Chemicals:

DPPH (1,1-diphenyl-2-picrylhydrazyl), ethanol, Ascorbic acid, Petroleum ether, Nutrient agar, DMSO,

Phosphate buffer, Sterile disc, Incubator. UV Spectrophotometer

Collection of plant material

Emblica officinalis and *Azadirachta indica* leaves were collected at Grace College of Pharmacy Konduthirappully, Palakkad in March 2023 and authenticated at Department of Botany by Dr. Suresh V. The collected leaves are shade dried and then made into course powder.

Preparation of plant extract

Individual leaves are extracted with ethanol by cold maceration procedure. In an electric water bath, the extract was centered and kept at 4°C for later use[4].

Test Organisms:

Grampositive and negative strains of bacteria, including *B. subtilis* and *E. coli*, were procured from the laboratory for microbiology at the Grace College of Pharmacy in Palakkad.

***In-vitro* antibacterial activity**

Preparation of inoculum

E. coli and *B. subtilis* were the bacteria employed in the antibacterial test. Gram staining and biochemical tests were used to ensure the purity of the microbial cultures, which were then cultivated in nutrient broth at 37°C and kept in nutrient agar at 2–8°C (3).

Disc diffusion technique(5)

The Petri plates are all set up, and they undergo a 15-minute autoclave at 121 °C. Under laminar air flow, they were permitted to cool. 20 ml of medium should be aseptically poured into each sterile Petri plate and allowed to harden. To ensure that the bacteria were distributed equally across the agar medium, 1 ml of inoculum suspension was dispersed over it using a sterile glass rod. A 50 mg/ml mixture of *Emblica officinalis* and *Azadirachta indica* leaf extract, Gentamycin, and DMSO as a control was introduced onto the readily prepared sterile disc. After the paper discs were positioned sufficiently apart on the medium and placed on the plates, they underwent incubation at 37°C for a 24-hour period. The antibacterial activity was assessed using area of inhibition measurements.

***In-vitro* antioxidant activity**

DPPH assay method (6)

The protective antioxidant property of a mixture of *Emblica officinalis* and *Azadirachta indica* leaf extract is scrutinised by the DPPH free radical scavenging assay technique. To formulate a DPPH solution, 50 ml of ethanol was mixed with 12.5 mg of DPPH. In order to prepare the stock solution for use, the absorbance has been decreased by ethanol to an absorbance of 0.98 before being kept. A sample of *Emblica officinalis* and *Azadirachta indica* leaf extract at different strengths (20, 40, 60, 80, and 100 µg/ml were prepared in ethanol) was combined with 1 ml of the DPPH solution. The reaction mixture was allowed to stand at 37 °C in complete darkness for 20 to 30 minutes. At 517 nm, the absorbance was calculated. Ascorbic acid was applied as a standard.

$$Q\ (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULT

The percentage yield of Amla leaf extract is 4.93% and Neem leaf extract is 4.04%.

***In vitro* antibacterial activity:**

It became clear that the ethanolic mixture of *E. officinalis* and *A. indica* leaf extract was potent against the bacteria *E. coli* and *B. subtilis*. For *Bacillus,* the zone of inhibition is at its largest, whereas for *E. coli*, it is at its lowest. In the investigation, the additive impact achieved through the combination of plant extracts against the tested microorganism was confirmed and visible.

***In vitro* antioxidant activity:**

The DPPH assay is implemented to assess in vitro antioxidant activity. The capacity of the mixture of ethanolic extracts to scavenge free radicals was concentration-dependent. The ethanolic plant extract exhibited synergistic action.

DISCUSSION

Due to the presence of alkaloids, flavanoids, tannins, and phenolic components in the previous investigations, the antibacterial activity of each separate ethanolic leaf extract of Amla and Neem was assessed. Leaf extract, when taken in combined form, showed additive activity against the tested pathogen (table-1 and fig-1, 2). Furthermore, this mixture of leaf extracts exhibited synergistic antioxidant activity (table-2 and fig-3).

Table.1:*In vitro* antibacterial property

ZONE OF INHIBITION(mm)			
SL.NO	**Treatment**	***E.coli***	***B.subtilis***
1	Control (DMSO)	10	10
2	Standard (Gentamycin)	35	35
3	*E,officinalis* leaf extract	13	14
4	*A.indica* leaf extract	19	20
5	Combined leaf extract	29	30

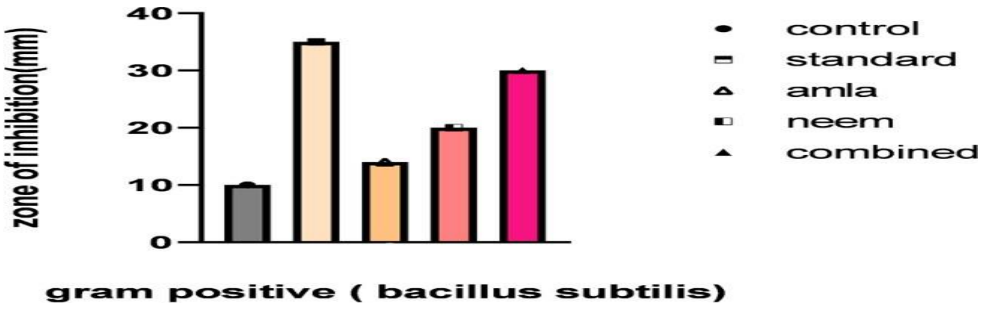

Fig-1 *In vitro* antibacterial property

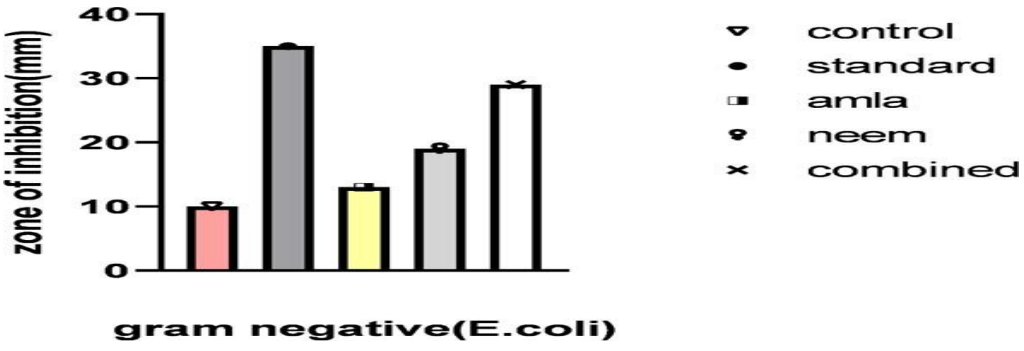

Fig-2*In vitro* antibacterial property

Table-2*In vitro* antioxidant activity

Concentration (µg/ml)	DPPH radical inhibition (%)			
	Ascorbic acid*	**Neem leaf extract***	**Amla leaf extract***	**Combined leaf extract***
20	48.97± 0.529	16.32± 0.10	14.28± 0.057	37.75± 0.577
40	54.08± 0.054	21.42± 0.071	18.36± 0.199	44.89± 0.127
60	66.32± 0.467	28.57± 0.157	23.46± 0.288	57.14± 0.389
80	86.73± 0.063	34.69± 0.093	36.73± 0.55	74.48± 0.299
100	94.89± 0.04	39.79± 0.421	35.71± 0.577	81.83± 0.577

***The values are mean± SEM (n=3)

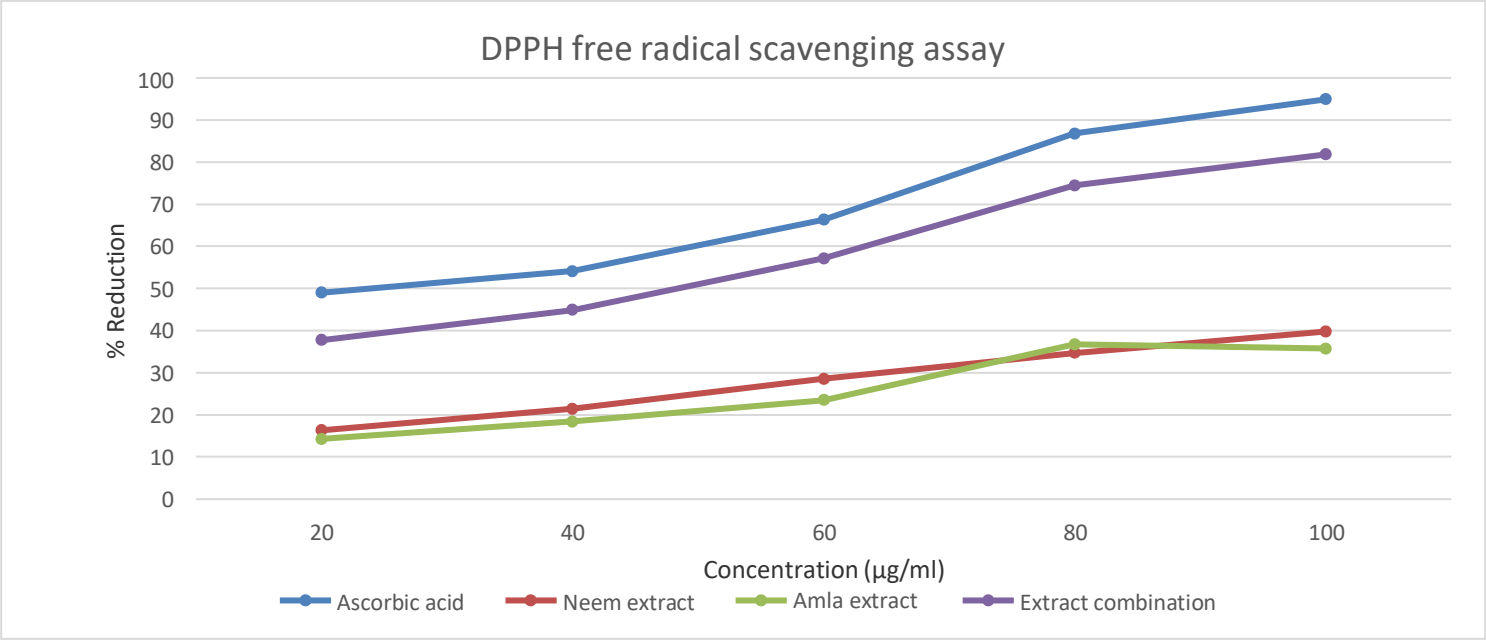

Fig-3 *In vitro* antioxidant activity

CONCLUSION

The present study revealed that the ethanolic mixture of *Emblica officinalis* and *Azadirachta indica* leaf extract posses additive antibacterial effect against *E.coli* and *B.subtilis* and shows synergistic antioxidant activity in dose dependent manner.

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