STUDIES ON PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF LEAF EXTRACT OF HYPTIS SUAVEOLENS (L.) POIT.

Arya Prabhakaran[1] and P. Prathyusha[2*]

[1]Department of Botany, Nirmala College for Women, Red fields, Coimbatore - 641 018

[2]Assistant Professor, Department of Botany, Nirmala College for Women, Red fields, Coimbatore – 641 018

*aryaprabhakaran437@gmail.com*prathyusha.phd@gmail.com +91 99943 99078*

ABSTRACT

Hyptis suaveolens (L.) Poit., an invasive weed species belongs to the family Lamiaceae is collected from Narimalamkunnu, a small hilly area located near Kumaranellur in Palakkad District, Kerala. Phytochemical analysis was done as per standard procedure. Antibacterial activity detected by using well diffusion method and antioxidant activity can be studied using the assays such as DPPH, FRAP and H_2O_2 radical scavenging activity. Methanolic extract of leaf of H. suaveolens shown the presence of carbohydrates, reducing sugars, proteins, amino acids, flavonoids, cardiac glycosides, saponins, phenolic groups, terpenoids, tannins and steroids. The antibacterial activities are studied by using four susceptible pathogens at different concentrations and significant activity was recorded. The methanol extract of leaf of H. suaveolens possess high radical scavenging activity also. We can suggest it as a potent indigenous drug for curing oxidative stress related diseases.

Key words: *Antibacterial activity, Antioxidant activity, Hyptis suaveolens, Phytochemicals*

1. INTRODUCTION

Plants have a significant role in the survival of all forms on earth. Either, being the source of food, fodder, energy etc., they are used as a source of medicine in curing several infections. The history of plants and medicine is as old as civilization and the origin of the earth. They play a critical part in the development of mortal societies around the whole world. Also, some plants consider as an important source of nutrition and as a result of that these plants are recommended for their remedial values. Although only a small chance of plant species has a specific mortal operation, multitudinous of them serve vital places in natural ecosystems and the services they

give. Rare plant species have remarkable characteristics that may be precious in future. Plant species can be estimated not only for their chemical mixes that are used as food by humans, analogous as carbohydrates, proteins, and lipids, but also for a wide range of chemicals that have physiological goods, analogous as alkaloids, glycosides, changeable oils, tannins, and so on.

Antibacterial agents are a group of paraphernalia that fight against pathogenic bacteria. Thus, by killing or reducing the metabolic exertion of bacteria, their pathogenic effect in the natural surroundings will be minimized. Antioxidants are mixes that can help or repair damage to body cells caused by oxygen by delaying or inhibiting the oxidation of lipids or other molecules by reducing the induction or propagation of oxidative chain responses [1]. As a result, antioxidants are being explored vastly in pharmacology, particularly as antidotes for neurological and renal ails, among other goods[2]. All-natural anti-oxidants, while safer, have lower antioxidant exertion than synthetic anti-oxidants. Still, because these are considered carcinogens, there is a need for safer, more cost-effective natural anti-oxidants with high antioxidant exertion. It has been reported that DPPH can be used as reagent to test the antioxidant exertion of small molecules, pure chemicals and plant extracts[3]. Family Lamiaceae (mint family) correspond of a group of plants with high medicinal value. It's a family of great diversity with smart distribution. Utmost of the species belonging to the family are sweet and retain essential oils. The sweet essential oils are mainly present in leaves. They are precious in cosmetic, flavouring, scent, perfumery, germicide and medicinal industriousness[4].

Hyptis suaveolens (L.) Poit. belonging to Lamiaceae family is a soft suffrutescent and ruderal weed that generally grows along the roadsides and the wet peripheries of ponds. The plant is native to tropical America but now distributed throughout the whole world from tropical to tropical regions Besides the dangerous effect of Hyptis suaveolens on natural ecosystems as aggressor weed, the plant is an important source of multitudinous pharmacological and artificial constituents.

2. MATERIALS AND METHODS

2.1 Plant Collection and preparation of plant extract

Leaves of *Hyptis suaveolens* (L) Poit was collected from Narimalamkunnu, a hilly area located near Kumaranellur village in Palakkad district in Kerala. Plant was shade dried and then the powdered. plant material 100 gm was extracted with 250 ml methanol by Soxhlet apparatus for 72 hours. Then the extract was stored in refrigerator until used for further analysis.

2.2 Preliminary Phytochemical Analysis

Phytochemical testing was performed to assess the various phytoconstituents present in methanolic extract of Hyptis suaveolens (L.) Poit. Qualitative analysis of its extract was performed to determine the presence or absence of carbohydrates, reducing sugars, proteins, amino acids, glycosides, alkaloids, flavonoids, saponin, terpenoids and steroids, tannins, phenolic compounds,

quinones and anthraquinone by Trease and Evans (1989)[5], Harborne (1973)[6], Brindha (1991)[7].

2.3 Antibacterial activity

Two strains of gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Pseudomonas aerogenosa*) were used in this study to show the antibacterial activity against the plant leaf extract. Antibacterial activity can be detected by the procedure mentioned in well diffusion method (Bauer *et al.,* 1996)[8].

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

The free radical scavenging activity of methanolic extract of leaves of *H. suaveolens* measured by using 2,2- diphenyl -1-picrylhydrazyl (DPPH). The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al.,* 2001). [9]. The percentage of inhibition of DPPH was calculated as:

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)=045}}{\text{A518 (control)}} \times 100$$

2.4.2 Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of plant extract samples was estimated according to the procedure described by Benzie and Strain (1996)[10] and as modified by Pulido *et al.,* (2000)[11]. Percentage of inhibition can be calculated as:

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)=0.422}}{\text{A518 (control)=0.459}} = 0.91 \times 100 = 91\%$$

2.4.3 Hydrogen peroxide scavenging activity

This activity of the plant was evaluated by the method of Ruch *et al.,* (1989)[12]. Percentage of inhibition can be calculated as:

$$\% \text{ Inhibition} = (\text{A control-A sample}) / \text{A control}) \text{ X } 100$$

3. RESULT AND DISCUSSION

3.1 Phytochemical analysis

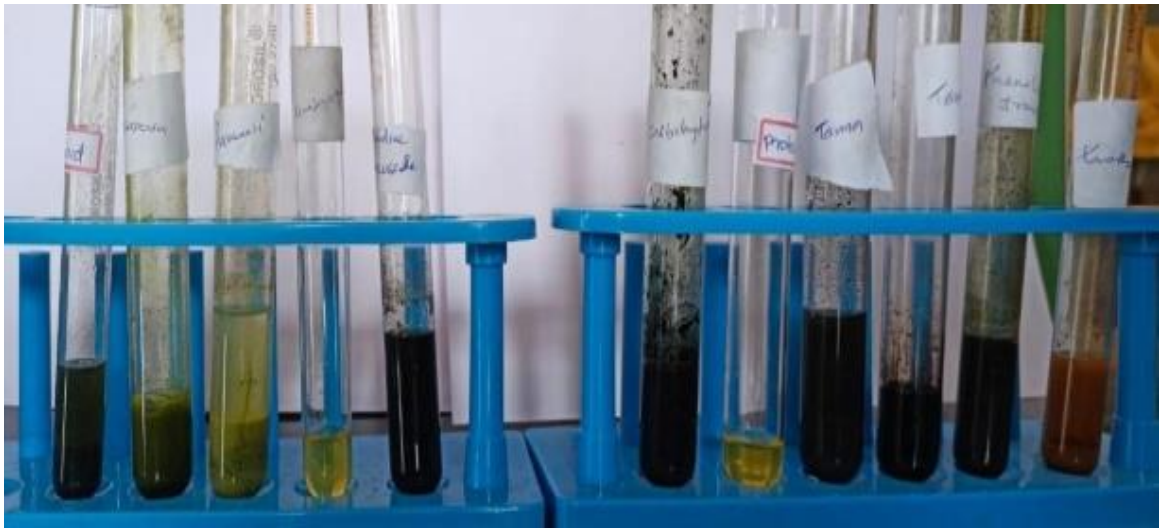
Table – 1: Result showing biochemical and phytochemical analysis

Phytochemicals	**Methanol extract of *H. suaveolens***
Carbohydrate	+++
Reducing sugar	+++
Protein	++
Amino acid	++
Alkaloid	-
Flavonoid	+++
Cardiac glycosides	++
Saponins	++
Phenolic group	++
Quinone	-
Anthraquinone	-
Terpenoids	+
Tannins	+++
Steroids	++

+++ High amount ++ moderate amount + less amount – absence.

"+"sign indicates the intensity of the metabolite present.

Fig. 1: Figure showing phytochemical analysis of *Hyptis suaveolens* (L.) Poit.



The results of qualitative analysis of methanolic leaf extract of *Hyptis suaveolens* (L.) Poit. Revealed the presence of carbohydrates, reducing sugars, proteins, amino acids, flavonoids, cardiac glycosides, phenolic groups, terpenoids, tannins and steroids. Alkaloids, quinone and anthraquinone were absent. (Table -1, Fig.1).

3.2 Antibacterial activity

Antibacterial activity was performed in well prolixity system by Van der watt *et al.*, (2001)[13]. The stock culture of bacteria (*Escherichia coli*, *Bacillus subtilis, Pseudomonas aerogenosa and Staphylococcus aureus)* were entered by enduing in nutrient broth media and grown at 37 for 18 hours. In this his disquisition, the active phytocomponents studied and further the antibacterial activity of the plant extract were analysed in well prolixity system Table-2 epitomized the bacterial growth inhibition of methanolic extract of the plant. The conformation of clear me around discs of plant extract and control indicate that the bacteria are sensitive and it's growth is inhibited by factory excerpt in these areas. The methanolic extract of *H. suaveolens* showed more antibacterial exertion 100 µl and 75 µl attention against *S. aureus* 15 cm and 2.0 cm independently. (Table-2). Zone of inhibition was veritably low in *E. coli* in all the attention. The methanolic excerpt was more active The inhibiting exertion may be pathogen specific or due to the phytochemical parcels of separate plant species and detergent used for the birth of secondary metabolites. The chemical element present in the plant are responsible for the antibacterial exertion.

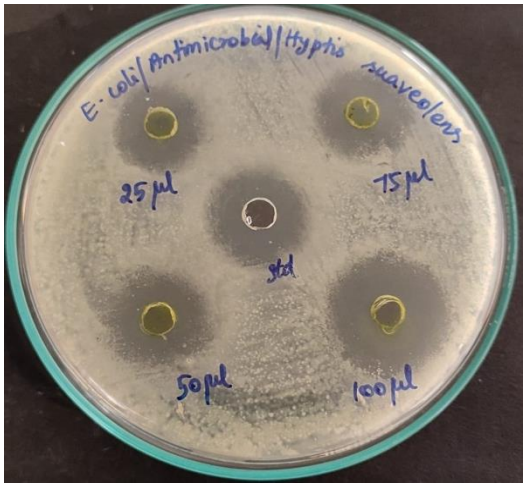
The results showed that the splint excerpt of Hyptis suaveolens is showing high antibacterial exertion when compared to standard Gentamycin. Gram positive bacteria show high vulnerability to *Hyptis suaveolens* excerpt than Gram negative bacteria. Prize show premier antibacterial exertion with maximum zone of inhibition values of 1.5 cm.2.0 cm.0.5 cm,1.8 cm

against *S. aureus* followed by zone of inhibition values 1.3 cm.1.5 cm.0.4 cm, 1.2 cm against *B. subtilis Hyptis suaveolens* extract show moderate exertion with zone of extract inhibition values 0,8 cm, 1.0 cm, 0.3 cm, 0.1 cm against *P. aerogenosa*. Smallest exertion observed against *E. coli* with 0.4 cm, 0.5 cm, 0.2 cm, 0.5 cm as zone of inhibition values, *H. suaveolens* excerpt show high exertion against *S. aureus* strains when compared to standard Gentamycin.

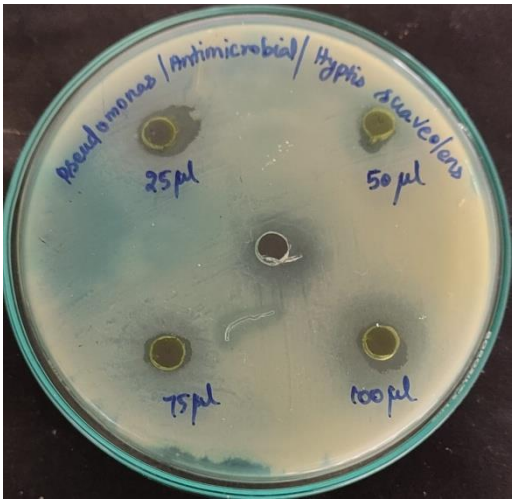
Table-2: Antibacterial activity of methanolic extract of *Hyptis suaveolens* against *E.coli, B. subtilis, P. aerogenosa and S. aureus.*

Name of the bacterial species	25 µl	50 µl	75 µl	100 µl
	Zone of inhibition (cm)			
E.Coli	0.5 cm	0.2 cm	0.5 cm	0.4 cm
P. aerogenosa	0.1 cm	0.3 cm	1.0 cm	0.8 cm
B. subtilis	1.2 cm	0.4 cm	1.5 cm	1.3 cm
S. aureus	1.8 cm	0.5 cm	2.0 cm	1.5 cm
Gentamycin	1.2 cm	0.6 cm	2.0 cm	1.6 cm

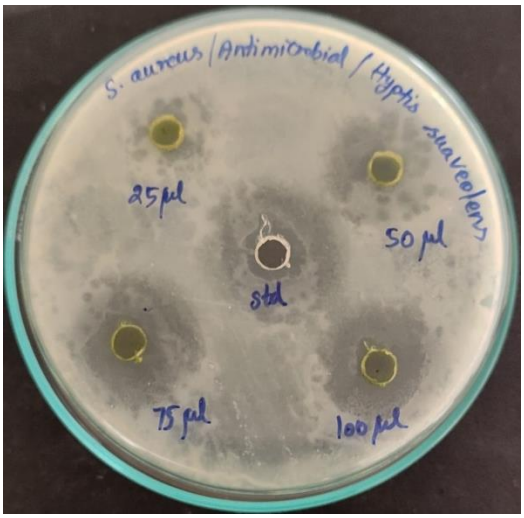
Fig.2: Antibacterial activity of methanolic extract of *H. suaveolens against E.coli, B. subtilis, P. aerogenosa* and *S. aureus*

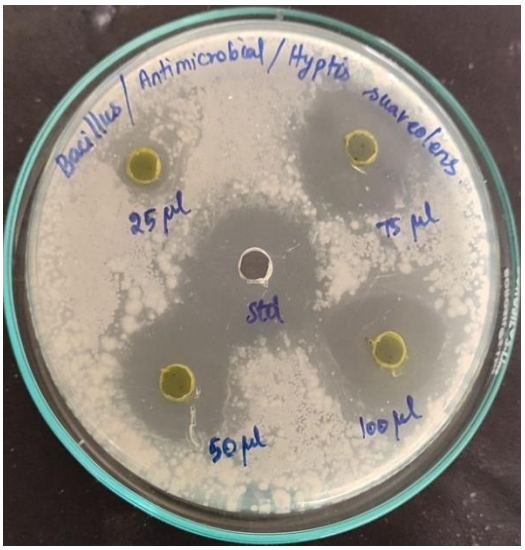


E.coli



P. aerogenosa





S. aureus ***B. subtilis***

3.3 Antioxidant activity

DPPH assay was used for evaluating the free radical scavenging action of *Hyptis suaveolens* (L.) Poit. In this assay, ascorbic acid was used as a standard compound. *Hyptis suaveolens* methanol extract showed minimum percentage of inhibition at 0.5 µl, i.e; 18.90% and maximum percentage of inhibition at 2.5 µl, i.e; 70.70% (Table-3, Fig.3), It shows that plant extract is a potent antioxidant and it is also found that a good correlation exists between the concentration of extract and percentage of inhibition. The percentage of inhibition of DPPH free radical increased with the increase of concentration of extract.

In FRAP assay, ascorbic acid was used as standard compound to determine antioxidant activity. It showed high percentage of inhibition at 2.5 µl, i.e; 73.02% and low percentage of inhibition at 0.5 µl, i.e., 25.07%. Here also result shows that plant extract is a potent antioxidant with increase in percentage of inhibition along with increase in concentration of extract (Table-4, Fig.4). In H_2O_2, radical scavenging activity, ascorbic acid is used as standard compound. % Inhibition were high at 2.5 µl, i.e., 78% and low at 0.5 µl, i.e., 14%. Here also percentage of inhibition increase with increase in concentration of extract (Table-5, Fig. 5).

Antioxidant activity of leaf extract of Hyptis suaveolens were evaluated by three assays; DPPH, FRAP and Hydrogen peroxide scavenging activity. It is based on transfer of reagent radical and measured by means of spectrophotometer. Hyptis suaveolens extract showed significant free radical scavenging activities against DPPH, FRAP, and H_2O_2 assays, when compared to that of standard ascorbic acid.

Table-3: DPPH radical scavenging activity of *Hyptis suaveolens*

Concentration	Standard (Ascorbic acid)	*Hyptis suaveolens*
0.5 µl	15 %	18.90 %
1.0 µl	38 %	35.12 %
1.5 µl	56 %	47.69 %
2.0 µl	73 %	60.27 %
2. 5 µl	86 %	70.70 %

Table-4: Ferric reducing/antioxidant power (FRAP) assay of *Hyptis suaveolens*

Concentration	Standard (Ascorbic acid)	*Hyptis suaveolens*
0.5 µl	33 %	25.07 %
1.0 µl	44 %	32.11 %
1.5 µl	52 %	52.19 %
2.0 µl	71 %	69.5 %
2. 5 µl	83 %	73.02 %

Table-5: Hydrogen peroxide scavenging activity (H_2O_2) activity of *Hyptis suaveolens*

Concentration	Standard (Ascorbic acid)	*Hyptis suaveolens*
0.5 µl	32 %	14 %
1.0 µl	48 %	36 %
1.5 µl	55 %	46 %
2.0 µl	72 %	68 %
2. 5 µl	87 %	78 %

Fig. 3 : DPPH radical scavenging activity of *Hyptis suaveolens*

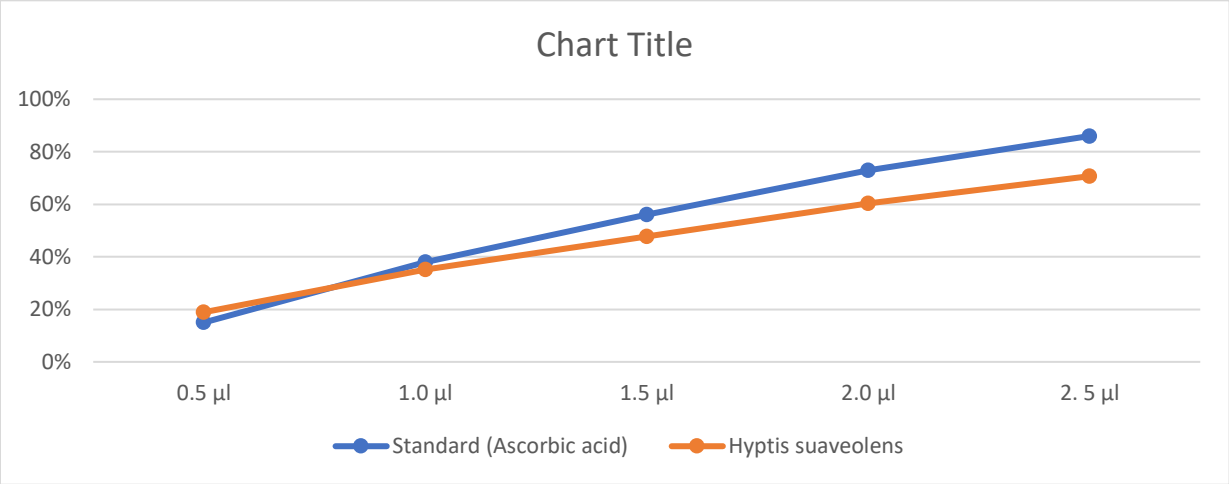


Fig. 4: FRAP Assay

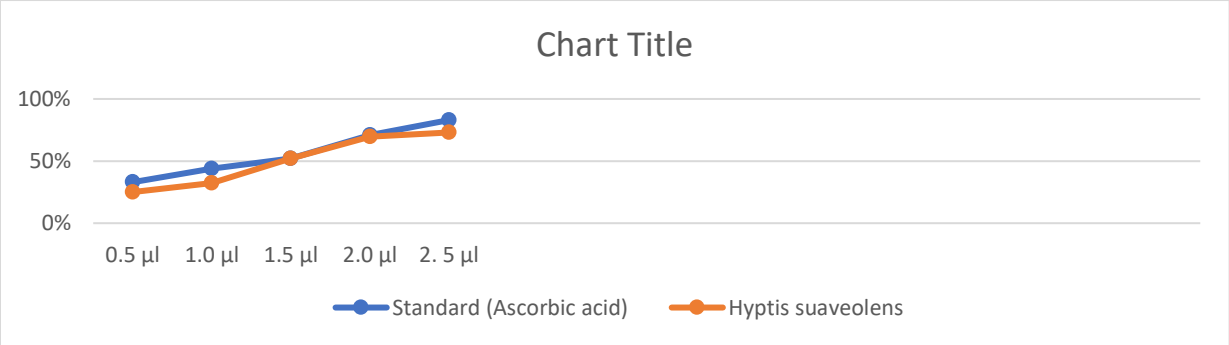
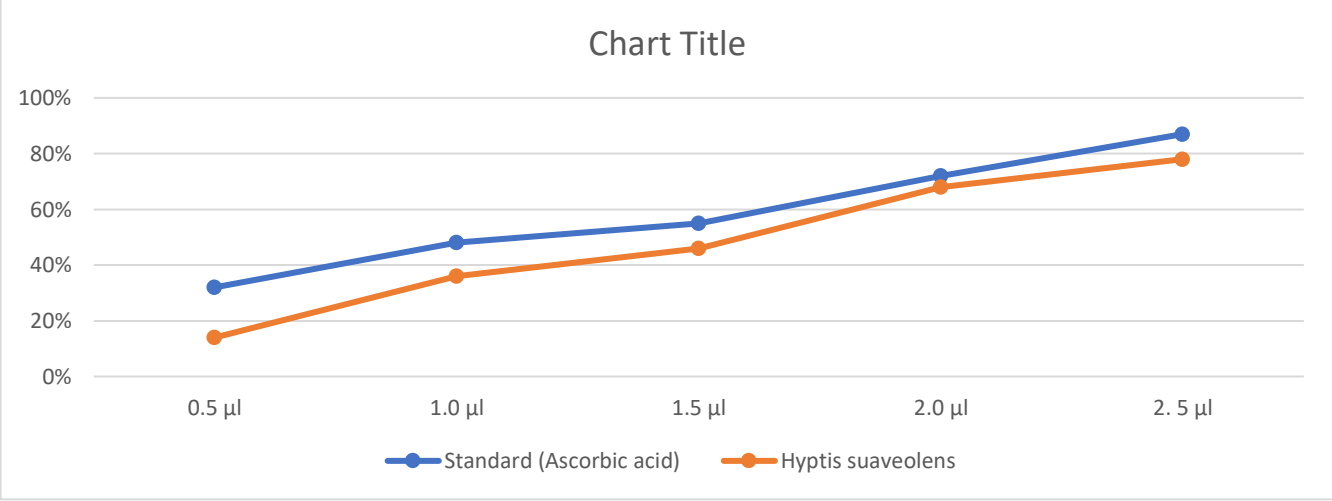


Fig. 5: Hydrogen peroxide radical scavenging activity



4. CONCLUSION

The methanolic splint excerpt of *Hyptis suaveolens* (L.) Poit contain carbohydrates, proteins, amino acids, flavonoids, cardiac glycosides, phenolic groups, terpenoids, tannins, steroids and saponin. Compliances confirm that the excerpt of H. suaveolens splint in respect to its antimicrobial exertion and the broad diapason of exertion makes it a promising indigenous medicine. The excerpt showed potent radical scavenging capability and the chance inhibition was plant directly commensurable to the increase in attention or chance of the factory excerpt.

The Phytochemical analysis revealed the presence of wide array of phytochemicals in *H. suaveolens.* The results easily indicated that the medicinal factory *H. suaveolens* retain strong antibacterial exertion against the pathogenic bacterial strains similar as *E.coli, B. subtilis, P. aerogenosa and S. aureus.* The excerpt may contain phytochemicals that beget inhibition substantially phenols and flavonoids. This property of factory may be important in precluding oxidative stress related conditions. There's good compass in examining the condiment for its antibacterial and antioxidant and free radical scavenging exertion in vivo.

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