Geno-Protective, Free Radical Scavenging and Antimicrobial Potential of *Hyptis suaveolens* Methanolic Fraction: An In-vitro Study

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Authors’ contributions

This work was carried out in collaboration among all authors. Author DI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ABD and MSK managed the analyses of the study. Author MSK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

**Aims:** *Hyptis suaveolens* (L.) Poit, is one of the natural herbs with several medicinal properties. However, many medicinal aspects of this herb still need to be explored. Therefore, our aim was to examine the antioxidant, antimicrobial properties and genoprotective effect of *H. suaveolens* methanolic extracts (HSME) of seed, stem, and root.

**Study design:** extraction and therapeutic aspects of *H. suaveolens*.

Place and Duration of Study: 1) Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Al-Majmaah and 2) Clinical Biochemistry & Natural Product research laboratory, Department of Biosciences, Integral University, lucknow between 2018-2020.

**Methodology:** HSME were extracted through soxhlet extractor and further analyzed for TPC, antioxidant activity through DPPH and FRAP assay followed by antimicrobial potential through
zone of inhibition and MIC/MBC assay. We also examined the genoprotective properties of HSME on oxidative DNA damage.

**Results:** Our results showed that TPC (180±5 mg GAE/g dw), DPPH scavenging activity (IC\textsubscript{50} value = 72±0.45 µg/ml) and FRAP value (1.443±0.02 µM ferrous ion/mg extract) was highest in HSME seeds followed by root and stem. The results also illustrated that the antimicrobial activity of HSME (seed and stem) against five bacterial strain were found very effective than root part. Moreover, genoprotective effect of HSME seeds (80±3 % retention) was better than stem (41±2 %) and root (32±2 %) extract.

**Conclusion:** The study revealed that HSME seed extract showed potential bioactivities might be due to presence of high TPC and can be used to treat diseases related with oxidative stress or microbial infections.

**Keywords:** DNA damage; hyptis suaveolens; methanolic fraction; microbicidal; oxidative stress.

1. INTRODUCTION

Oxidative stress is responsible for the development of cell injury, aging, cardiovascular diseases, gastrointestinal infection, neurodegenerative diseases, kidney disorders and cancer. Overproduction of free radicals which can also induce oxidative stress leads to the damage to lipids, nucleic acid, and other biomolecules [1,2]. Microbial infections can also damage biomolecules, induce oxidative stress, and are involved in pathogenesis of various diseases [3], such as periodontal diseases, inflammation, Parkinson’s disease [4,5].

Microbes develop the resistance mechanism due to overuse of antibiotics. Therefore, it has become a global concern which required novel findings to treat microbial infection [6,7]. Certain large number of synthetic drugs have been developed against microbial infections and oxidative stress having various side effects, toxicity and uneconomical in long term use [7,8]. Therefore, it is necessary to develop novel drugs from natural resources because of its high availability, cost affectivity, low toxicity and of having least side effects in prolonged use [9].

Previously, it has been reported that plants are the good source of natural bioactive compounds against vast variety of diseases [10]. Medicinal plants or their bioactive fractions were also reported as potent antioxidant, antimicrobial, geno-protective, hypolipidemic, anti-diabetic, neuroprotective agents [11-23]. Studies also revealed the antioxidant and antimicrobial effects of several plant species, such as *Nandina domestica* [24]. Previously, plant species of *Lamiaceae* family have been reported for their medicinal properties, such as *Hyptis suaveolens*, *Ocimum tenuiflorum* L. and *Ocimum basilicum* L [25,26,27].

![Fig. 1. H. suaveolens](https://flora.indianbiodiversity.org/flora/angiosperm/lamiales/lamiaceae/hyptis/hyptis-suaveolens)

*Hyptis suaveolens* (L.) Poit (*Lamiaceae*), is a rigid sweetly aromatic herb well known for its insecticidal activity, against *Anopheles gambiae* [28], *Drosophila melanogaster* and *Artemia salina* [29]. *H. Suaveolens* (Fig. 1) have also been reported for its potent antimicrobial, antiplasmodial and antioxidant properties [27,28,30,31]. Various bioactive compounds were isolated from essential oils of *H. suaveolens* [29]. Moreover, bioactive fractions of *H. suaveolens* have shown decreased neurotoxicity [32], hypoglycemic [33] and anticancer [34] properties. Seeds and leaves extract of *H. suaveolens* were reported to control Trogoderma granarium Everts in stored groundnut [35].

However, there is no report on *H. suaveolens* methanolic extracts (HSME) of seed, stem, and root for their antioxidant, antimicrobial and
genoprotective properties. Specified the fact of traditional knowledge and the recent pharmacological studies for *Hyptis suaveolens*, the aim of the present study was to evaluate the phytochemical, DPPH free radical scavenging activity, FRAP value, Geno-protective potential, and antimicrobial properties against gram positive (*Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus cereus*) and gram negative (*Enterobacter aerogenes* and *Klebsiella pneumoniae*) microbes of HSME (seed, stem, and root).

2. MATERIALS AND METHODS

2.1 Chemicals

All reagents and chemicals were of analytical reagent grade and procured from Sigma Aldrich. Culture media was procured from Himedia Laboratory Pvt. Ltd.

2.2 Extract Preparation

Different parts (seeds, stem, and root) of *H. suaveolens* were collected from neighboring area of Lucknow near Integral University, washed with distilled water and shade dried for 15 days. Plant materials then pulverized using an electric grinder. The extractions of 20 gm powder with methanol (200 ml) were done using soxhlet extractor for about 8-10 hours [36]. The extracts were then concentrated using rotatory evaporator and placed at -20°C till use.

2.3 Phytochemical Analysis

The freshly prepared crude extracts were qualitatively analyzed for the presence of phytochemicals as described by Harborne [37].

2.4 Estimation of Total Phenolic Content

Total phenolic content (TPC) of HSME root, stem and seed was determined by using Folin-Ciocalteu (F-C) reagent and is expressed as gallic acid equivalent (GAE) in µg/mg dry weight of extract [38].

2.5 DPPH Radical Scavenging Property Assay

The DPPH assay was performed according to the method described by Brand-Williams [39], with some modifications [20]. The scavenging property was calculated as IC$_{50}$ value based on the percentage of DPPH radical scavenged using the following equation:

\[
\% \text{ scavenging effect} = \left[ \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \right] \times 100
\]

IC$_{50}$ value is the efficient concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid standard was used as positive control.

2.6 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay of HSME (seeds, stem, and root) was done by following the procedure of Benzie and Strain [40], with a few modification [21]. The standard curve was plotted using ferrous sulphate solution, and results were expressed as μM Fe(II) /mg dry weight of extract.

2.7 DNA Protection Assay

Oxidative DNA damage by Fenton’s reagent was measured on pUC18 plasmid DNA, according to the procedure described by Iqbal et al. [21]. Briefly, reaction mixture (RM) was prepared by adding 10 μl of HSME of seed, stem, and root at different concentrations (50 & 100 μg) and 1 μl (100 ng) plasmid DNA were incubated for 10 min at 27°C temperature followed by the adding up of 10 μl Fenton’s reagent (30 mM H$_2$O$_2$, 50 μM Ascorbic Acid & 80 μM FeCl$_3$) in 0.5 ml micro centrifuge tubes. This reaction mixture was then run through electrophoresis (1% agarose) and protection of DNA damage by plant extracts were analyzed followed by ethidium bromide (EtBr) staining with Gel Doc XR system (Bio-Rad, USA). Mannitol (10 μg/RM) was used as standard in this assay.

2.8 Antimicrobial Activity Assay

Bacterial strains (*Staphylococcus epidermidis* (NCIM 2493), *Bacillus subtilis* (NCIM 2920), *Bacillus cereus* (NCIM 2156), *Enterobacter aerogenes* (NCIM 5139) and *Klebsiella pneumoniae* (NCIM 2957)) were obtained from NCL (National Chemical Laboratory) Pune, India. For antibacterial testing fresh inoculums was prepared for each bacterial strain and incubated at 37°C for 24 h. To obtain turbidity comparable to that of McFarland 0.5 standard (1.5×10$^8$ cells/ml) the cells suspension was adjusted with nutrient broth according to Jorgensen HJ [41]. The antibacterial assays of the HSME of different parts (seeds, stem, and root) were carried out by means of agar well diffusion method [42].
Hundred micro liters (µl) of diluted inoculums (1.5 ×10^6 CFU/ml) of bacteria strain was swabbed over Nutrient agar (pH 7.2) plates. Wells of 4 mm diameter were punched into agar plate and filled with 40 µl of extract prepared in DMSO at different concentrations (10-50 µg/ml). The plates were left for 30 min at 27°C temperature to allow the diffusion of the extract and then incubated at 37°C for 18 h. The diameter of inhibition zone was measured in millimeters including the size of the wells. DMSO without extract was used as a control and antibiotics such as Penicillin and Tetracycline having potency of 10 µg per disc were used as standard.

2.9 Analysis of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) was analyzed for each plant extract showing antimicrobial activity against test pathogens [43]. Plant extracts were re-suspended in DMSO to prepare 5 mg/ml final concentration as stock and then 5-fold serial dilutions were done; added to broth media of 96-well microtiter plate. Thereafter, 100 µl inoculums (1×10^6 CFU/ml) were added to each well. This microtiter plate was incubated at 37°C for 24 h. Each plant extract was assayed in triplicate and each time two sets of microtiter plate were prepared; one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC was defined as the lowest concentration preventing visible growth. The MBC of the HSME was determined as described by Mishra et al. [44]. The plates of MIC, which showed no visible growth, were cultured on fresh nutrient agar plates. The lowest concentration of antimicrobial agent/plant extract, from which bacteria do not recover on fresh medium, was treated as MBC.

2.10 Statistical Analysis

For all protocols, samples were analyzed in triplicate and the results were expressed as mean ± SD. Fifty percent inhibitory concentration (IC_{50}) were calculated by Origin version 6.0 Professional software (Origin Lab Corporation, Northampton, MA, USA), and the results were evaluated using one-way analysis of variance (ANOVA) and two tailed Student’s t-test. Statistical significance was expressed as *p<0.05, **p<0.01.

3. RESULTS

3.1 Phytochemical Analysis

Qualitative phytochemical analysis represented in Table 1, reveals that HSME of stem is rich in flavonoids, terpenoids and proteins. Meanwhile, HSME of seeds is abundant only with phenol and scarcity of terpenoid was reported. However, HSME of root is only abundant with flavonoids.

3.2 Total Phenolic Content

Our results show that total phenolic content (TPC) of various HSME fractions was found to be in the following decreasing order: seeds > root > stem. The result presented in Table 2, clearly demonstrated that HSME of seed have better phenolic content (180 ± 3.72 µg GAE/mg of dry plant extract) than other plant parts like roots and stem (100 ± 2.96 and 82 ± 2.64 µg GAE/mg of dry plant extract, respectively).

3.3 In-vitro Antioxidant Activity

HSME (seeds, stem, and root) were screened for their in-vitro antioxidant properties by DPPH and FRAP analysis method. We found that HSME (seed) showed better DPPH radical scavenging activity (IC_{50} value = 72±0.45 µl/ml) than HSME of stem (>250±5.46 µg/ml) and root (143±2.15 µg/ml), respectively Table 2 and Fig. 2. Whereas, our results also showed in Table 2, that seeds of HSME have higher FRAP value (1.443±0.02 µM ferrous ion/mg extract) than the stem (0.367±0.004 µM ferrous ion/mg extract) and root (0.513±0.01 µM ferrous ion/mg extract) of HSME, respectively.

3.4 Geno-protective properties of HSME

Our results (Table 3) depicted that seed extract of H. suaveolens showed better DNA protection activity as represented in terms of retention percentage (35% ±2 at 50 µg/RM and 80% ±3 at 100 µg/RM) than stem (21% ±1 at 50 µg/RM and 41% ±2 at 100µg/RM) and roots (15% ±2 at 50 µg/RM and 32% ±2 at 100 µg/RM) of HSME, which is in accordance with free radical scavenging activity of HSME (seed).

3.5 Antimicrobial Activity

The H. suaveolens extract showed significant antimicrobial activity against different bacterial strains. The antimicrobial activity of methanolic extracts of H. suaveolens (seed, stem, and root)
and standard drugs against five bacterial strain 
\textit{B. cereus}, \textit{E. aerogens}, \textit{K. pneumoniae}, \textit{B. substilus}, \textit{S. epidermidus} at different concentration (0.1-5 mg/ml) were evaluated. Our results elaborated Fig. 3, 4, 5 that, HSME of seeds showed better activity against gram negative bacteria (\textit{E. aerogens}, \textit{K. pneumoniae}), than stem and root parts. Whereas HSME of stem showed better potency against gram positive bacteria (\textit{B. substilus} and \textit{B. cereus}) than seeds and root part. HSME of root part was found to be more effective against \textit{S. epidermidus} (gram positive).

Among standard drugs, penicillin showed lower activity than tetracycline against all tested microbial pathogens Fig. 6. MIC and MBC results Table 4 were found to be in correlation with zone of inhibition assay for antimicrobial activity of plant extract. MIC and MBC for HSME of seeds against \textit{E. aerogens} and \textit{K. pneumoniae} were reported better than root and seeds extract. Whereas stem extract showed higher potency of MIC and MBC against \textit{B. substilus} and \textit{B. cereus} than seeds and root part whereas root part is more effective against \textit{S. epidermidus}.

\textbf{Table 1.} Phytochemical screening of HSME of seeds, stem, and root

| Plant part | Flavonoids | Glycosides | Reducing sugar | Tannins | Terpenoids | Phenol | Proteins |
|------------|------------|------------|----------------|---------|------------|--------|----------|
| HSME (seed)| +          | +          | +              | +       | +          | ++     | +        |
| HSME (stem)| ++         | +          | +              | ++      | ++         | --     | ++       |
| HSME (root)| ++         | +          | +              | ++      | ++         | ++     | ++       |

\textbf{Table 2.} TPC, FRAP value and DPPH radical scavenging activity of HSME seeds, stem, and root. Each value in the table is represented as mean ± SD (n = 3)

| Plant extracts | TPC (µg GAE/mg of dry plant extract) | FRAP µM ferrous ion/mg extract | DPPH assay (IC$_{50}$ value in µg/ml) |
|----------------|-------------------------------------|--------------------------------|-------------------------------------|
| HSME (seed)    | 182±3.72                           | 1.443±0.02                     | 72±0.45                             |
| HSME (stem)    | 82±2.64                            | 0.367±0.004                    | >250±5.46                           |
| HSME (root)    | 100±2.96                           | 0.513±0.01                     | 143±2.15                            |
| Ascorbic acid  | ----                                | ----                           | 15±0.89                             |

\textbf{Fig. 2.} DPPH free radical percentage scavenging activity of methanol extract of \textit{Hyptis suaveolens} [HSME] seed, stem, root extract at various concentrations. Each value in the figure is represented as mean ± SD (n = 3). Non-significant (ns), significantly different *P<0.05, **P<0.01 vs 0 µg/ml
Table 3. Retention percentage of supercoiled pUC18 plasmid DNA in hydroxyl radical-mediated in-vitro systems with extracts of *H. Suaveolens*.

| Concentration/Sample | 00 µg/RM | 10 µg/RM | 50 µg/RM | 100 µg/RM |
|----------------------|----------|----------|----------|-----------|
| Control              | 10±1     |          |          |           |
| HSME (seed)          |          | 35±2     | 80±3     |           |
| HSME (stem)          |          | 21±1     | 41±2     |           |
| HSME (root)          |          | 15±2     | 32±2     |           |
| Mannitol             |          |          |          | 90±3      |

Fig. 3. Zone of inhibition (mm) at various concentrations (mg/ml) for HSME of seed against bacterial strains. Each value in the figure is represented as mean ± SD (n = 3)

Fig. 4. Zone of inhibition (mm) at various concentrations (mg/ml) for HSME of stem against bacterial strains. Each value in the figure is represented as mean ± SD (n = 3)
4. DISCUSSION

4.1 Phytochemical Analysis

Phytochemicals are the rich source of plant derived medicinal drugs having antioxidant, antimicrobial properties and can be used to cure various diseases [13,19,24,45]. Previous studies revealed that aerial parts, roots, and essential oils of *H. suaveolens* possess medicinal properties might be due to prodigious number of phytochemicals [29,31,34]. Our qualitative study (Table 1) elaborated the presence of phytochemicals in seed, stem, and root of HSME which exhibited the presence of flavonoids, glycoside, reducing sugar, tannins, and proteins with abundance of phenol, however lower terpenoids were found in HSME seeds. HSME root was high in flavonoids although scarcity of tannins and phenols. HSME stem contains most of the phytochemicals with high in flavonoids content.
4.2 Total Phenolic Content

The Folin–Ciocalteu assay, recognized as one of the standard antioxidant testing procedures, measures the level of total phenolic content in natural products. Phenolic compounds are the major plant secondary metabolites with antioxidant activity [46]. In the present study, as part of analysis of chemical composition, total phenolic contents of seeds, stem, and root of HSME were determined. Our results illustrate the total phenolic content (TPC) of various fractions of HSME which were found to be in the following decreasing order: seeds > root > stem. The result presented in Table 2, clearly demonstrated that HSME of seed have better phenolic content (180 ± 3.72 µg GAE/mg of dry plant extract) than other plant parts like roots and stem (100 ± 2.96 and 82 ± 2.64 µg GAE/mg of dry plant extract, respectively). Furthermore, the existence of considerable amount of TPC in seeds of HSME is in agreement with prior phytochemical information on different parts of H. suaveolens which have phenolic compounds as major components [32].

4.3 In-vitro Antioxidant Activity

Excessive free radical generation leads to the oxidative stress which results in macromolecule damage and progression of various diseases [1,2]. Therefore, it is necessary to scavenge these free radicals to balance the homeostasis. Most common protocol to evaluate the free radical scavenging properties of plant extracts uses DPPH as free radical and FRAP assay for antioxidant potential [20,21,32,47]. HSME (seeds, stem, and root) were screened for their in-vitro antioxidant properties by DPPH method. We found that HSME (seed) showed better scavenging activity (IC₅₀ value = 72±0.45 µg/ml) than HSME of stem (>250±5.46 µg/ml) and root (143±2.15 µg/ml), respectively Table 2 and Fig. 2). Whereas, at low pH of about 3.6, reduction of ferric tripyridyl triazine (Fe3+ -TPTZ) complex to blue colored Fe2+ -TPTZ takes place, which has absorbance at 593 nm. Our results showed in Table 2, illustrated that seeds of HSME have higher FRAP value (1.443±0.02 µM ferrous ion/mg extract) than the stem (0.367±0.004 µM ferrous ion/mg extract) and root (0.513±0.01 µM ferrous ion/mg extract) of HSME, respectively.

The higher DPPH scavenging activity of HSME (seeds) might be due to its high phenolic content as TPC is directly related with antioxidant properties. Our results are in correlation with previous studies where the antioxidant potential of aerial parts and essential oils of H. suaveolens were illustrated [31,32]. Biological properties of H. suaveolens have been investigated in numerous studies and the results indicate that the reported therapeutic properties are mainly due to the presence of antioxidants compound [32,33]. Our findings are similar with the previous reports on other plants [20,21,48], where it is represented that antioxidant profile of plant extracts are significantly correlated with their total phenol content.

4.4 Geno-Protective Properties of HSME

DNA is a genetic material which controls all the metabolic process of living system. Oxidative stress induced highly reactive free radical species OH• through Fenton’s reagent, are prone to cause damage of plasmid supercoiled DNA to single stranded or nicked circular form [49]. It is apparent from our results Table 3 that the mixing of H. suaveolens extract at the concentration of 50 µg/10µl, 100 µg/10µl to reaction mixture protects DNA of pUC18 may be due to scavenging of the OH• radicals generated through Fenton’s reaction. HSME of seeds showed better reversal (80%) of oxidative DNA damage to its supercoiled form at 100 µg/RM

Table 4. The MIC and MBC values (mg/ml) of HSME (seed, stem, and root) against bacterial strains. The results are shown as average values from three separate experiments.

| Sample/Stains | B. cereus | E. aerogens | K. pneumonia | B. subtilis | S. epidermidis |
|--------------|-----------|-------------|--------------|------------|--------------|
|              | MIC       | MBC         | MIC          | MBC        | MIC          | MBC          |
| HSME (seed)  | 0.07±0.4  | 1±0.0       | 0.06±0.8     | 5±0.0      | 0.07±0.8     | 5±0.0        |
| HSME (stem)  | 0.04±0.5  | 0.5±0       | 0.4±0.0      | >5         | 0.19±0.5     | >5           |
| HSME (root)  | 0.09±0.7  | 3±0.0       | 0.08±0.2     | >5         | 0.08±0.4     | >5           |

Table 4: The MIC and MBC values (mg/ml) of HSME (seed, stem, and root) against bacterial strains. The results are shown as average values from three separate experiments.
Previously, it has been reported that scavenging of hydroxyl free radical have its impact on genoprotective properties, where it was observed that *Ficus virens*, *Aegle marmelos* and *Ficus palmata* extracts were reported to restrain the OH· dependent break of plasmid DNA [20,21,50].

### 4.5 Antimicrobial Activity

Appearance of multi-drug resistance in pathogenic microbes as well as adverse side effects of certain antibiotics has triggered enormous interest in the search for novel antimicrobial drugs especially from the natural resources [6,9]. Various plant species were analyzed for antimicrobial efficacy to compensate the resistant drugs for microbial pathogens [11,24,48]. *Lamiaceae* family contains good biological activity, such as *H. suaveolens* [31,32].

Therefore, antimicrobial activity of the *H. suaveolens* extracts and their effectiveness was quantitatively assessed by the presence or absence of zone of inhibition and scaling their diameter. The antimicrobial activity in this study was determined by using agar well diffusion assay, MIC, and MBC. The *H. suaveolens* extract showed significant antimicrobial activity against different bacterial strains. HSME of Seeds showed better activity against gram negative bacteria (*E. aerogens*, and *K. pneumoniae*), than stem and root parts.

We found the antimicrobial activity of HSME of seeds in the following decreasing order: *B. cereus* > *E. aerogens* > *K. pneumoniae* > *S. epidermidius* > *B. subtilis*. Whereas activity of HSME of stem in the following decreasing order: *B. cereus* > *B. subtilis* > *S. epidermidius* > *K. pneumoniae* > *E. aerogens* and HSME root is in the following decreasing order: *B. cereus* > *S. epidermidius* > *E. aerogens* > *K. pneumoniae* > *B. subtilis*. MIC and MBC results Table 4 were found to be in correlation with zone of inhibition assay for antimicrobial activity of plant extract. Similar results were found in previous studies where essential oils of *H. suaveolens* leaves, seeds showed antibacterial activity against gram-positive or gram-negative bacteria [26,27].

The study exposed that HSME of seeds exhibited potential bioactivities which might be due to presence of high TPC and can be used to treat ailments related with oxidative stress or microbial infections. Our study has some limitations such as only one microbial strain from each genus was analyzed for antimicrobial assays, and purification of bioactive compounds was not done. Therefore, further research can be done to check the potency of pure compounds of HSME of seeds on different microbial strains.

### 5. CONCLUSION

This prelude screening is an interesting assessment of the potential antimicrobial, DNA protective and antioxidant activity of *H. suaveolens*. On the light of these experiments, it could be concluded that the methanolic extracts of different parts of *H. suaveolens* exhibited a fascinating antibacterial activity against most of the strains tested. Study showed that methanolic extract of the *H. suaveolens* investigated are rich in flavonoids, tannins, phenols, reducing sugar, terpenoids and showed presence of glycosides. The methanol extract of *H. suaveolens* seeds showed maximum phenolic content, which in turn revealed maximum scavenging potential and hence also showed better genoprotective potential in comparison to stem and root extracts. The seed methanolic extract of *H. suaveolens* possess strong antioxidative activity. The occurrence of antioxidants is a desirable aspect which may have helpful health effect on anticipation of many diseases. Further studies require to be carried out to describe active principle(s) of fractions and to study the relation among chemical structure and antioxidant activity in vitro and in vivo and the mechanism by which *H. suaveolens* extract exhibit pharmacological actions.

### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

### CONSENT

It is not applicable

### ETHICAL APPROVAL

It is not applicable
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev; 2017.
2. Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem. 2017;86:715-48.
3. Huycke MM, Abrams V, Moore DR. Enterococcus faecalis produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. Carcinogenesis. 2002;23(3):529-36.
4. Mira A, Simon-Soro A, Curtis MA. Role of microbial communities in the pathogenesis of periodontal diseases and caries. J Clin Periodontol. 2017; 44: S23-38.
5. Qian Y, Yang X, Xu S, Wu C, Qin N, Chen S, Xiao Q. Corrigendum: Detection of Microbial 16S rRNA Gene in the Blood of Patients with Parkinson’s Disease. Front Aging Neurosci. 2019;11:4.
6. Sommer MO, Munck C, Toft-Kehler RV, Andersson DI. Prediction of antibiotic resistance: time for a new preclinical paradigm? Nat Rev Microbiol. 2017;15(11):689.
7. EFSA. Panel on food additives and nutrient sources added to food (ANS); scientific opinion on the reevaluation of butylatedhydroxyanisole-BHA (E 320) as a food additive. EFSA J, 2011;9(10):49. DOI: http://dx.doi.org/10.2903/j.efsa.
8. Tahir S, Mahmood T, Dastgir F, Haq IU, Waseem A, Rashid U. Design, synthesis and anti-bacterial studies of piperazine derivatives against drug resistant bacteria. European journal of medicinal chemistry. 2019;166:224-31.
9. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016;79(3):629-61.
10. Selamoglu Z. Traditional Medicine & Clinical Naturopathy. Pharm Res. 2018;16:92-8.
11. Ahmad J, Khan S, Iqbal D. Evaluation of antioxidant and antimicrobial activity of Ficus carica leaves: an in vitro approach. J Plant Pathol Microbiol. 2013;4:157.
12. Ahmad N, Bhatnagar S, Saxena R, Iqbal D, Ghosh AK, Dutta R. Biosynthesis and characterization of gold nanoparticles: Kinetics, in vitro and in vivo study. Materials Mater. Sci. Eng. C. 2017;78:553-64.
13. Alvi SS, Iqbal D, Ahmad S, Khan MS. Molecular rationale delineating the role of lycopene as a potent HMG-CoA reductase inhibitor: in vitro and in silico study. Nat. Prod. Res. 2016;30(18):2111-4.
14. Alvi SS, Ahmad P, Ishrat M, Iqbal D, Khan MS. Secondary Metabolites from Rosemary (Rosmarinus officinalis L.): Structure, Biochemistry and Therapeutic Implications Against Neurodegenerative Diseases. InNatural Bio-active Compounds. Springer, Singapore. 2019;1-24.
15. Akhter F, Hashim A, Khan MS, Ahmad S, Iqbal D, Srivastava AK, Siddiqui MH. Antioxidant, α-amylase inhibitory and oxidative DNA damage protective property of Boerhaavia diffusa (Linn.) root. S Afr J Bot. 2013;88:265-72.
16. Akhter F, Alvi SS, Ahmad P, Iqbal D, Alshehri BM, Khan MS. Therapeutic efficacy of Boerhaavia diffusa (Linn.) root methanolic extract in attenuating streptozotocin-induced diabetes, diabetes-linked hyperlipidemia and oxidative-stress in rats. Biomed Res Therapy. 2019;6(7):3293-3306.
17. Iqbal D, Khan A, Ansari IA, Khan MS. Investigating the role of novel bioactive compound from Ficus Virens ait on cigarette smoke induced oxidative stress and hyperlipidemia in rats. Iran J Pharm Res. 2017;16(3):1089.
18. Iqbal D, Khan MS, Khan A, Ahmad S. Extenuating the role of Ficus virens ait and its novel bioactive compound on antioxidant defense system and oxidative damage in cigarette smoke exposed rats. Biomed Res Therapy. 2016;3(07):723-32.
19. Iqbal D, Khan MS, Khan MS, Ahmad S, Hussain MS, Ali M. Bioactivity guided fractionation and hypolipidemic property of a novel HMG-CoA reductase inhibitor from Ficus virens ait. Lipids Health Dis. 2015; 14(1):15.
20. Iqbal D, Khan MS, Khan A, Khan M, Ahmd S, Srivastava AK, Bagg P. In vitro screening for ω-hydroxy-β-methylglutaryl-coa reductase inhibitory and antioxidant activity of sequentially extracted fractions of Ficus palmate forsk. Bio Med Res Int. 2014a;2014.
21. Iqbal D, Khan MS, Khan MS, Ahmad S, Srivastava AK. An in vitro and molecular informatics study to evaluate the antioxidative and ω-hydroxy-β-methylglutaryl-CoA reductase inhibitory property of Ficus virens ait. Phytother Res. 2014b;28(6):899-908.
22. Nabi R, Alvi SS, Shah A, Chaturvedi CP, Iqbal D, Ahmad S, et al. Modulatory role of HMG-CoA reductase inhibitors and ezetimibe on LDL-AGEs-induced ROS generation and RAGE-associated signalling in HEK-293 cells. Life Sciences. 2019;116823.
23. Khatoon A, Khan F, Ahmad N, Shaikh S, Rizvi SM, Shailk S, et al. Silver nano particles from leaf extract of Mentha piperita: Eco-friendly synthesis and effect on acetylcholinesterase activity. Life sciences. 2018;209:430-4.
24. Bajpai VK, Park I, Lee J, Shukla S, Nile SH, Chun HS, et al. Antioxidant and antimicrobial efficacies of a biflavonoid, amentoflavone from Nandina domestica in vitro and in minced chicken meat and apple juice food models. Food chem. 2019;271:239-47.
25. Piras A, Gonçalves MJ, Alves J, Falconieri D, Porcedda S, Maxia A, et al. Ocimum tenuiflorum L. and Ocimum basilicum L., two spices of Lamiaceae family with bioactive essential oils. Ind Crops Prod. 2018;113:89-97.
26. Asekun OT, Ekundayo O, Adeniyi BA. Antimicrobial activity of the essential oil of Hyptis suaveolens leaves. Fitoterapia. 1999;70(4):440-2.
27. Bachheti RK, Rai I, Joshi A, Satyan RS. Chemical composition and antimicrobial activity of Hyptis suaveolens Poit. seed oil from Uttarakhand state, India. Orient Pharm Exp Med. 2015;15(2):141-6.
28. Ivoke N, Okafor FC, Owoicho LO. Evaluation of ovicidal and larvicidal effects of leaf extracts of Hyptis suaveolens (L.) poit (Lamiaceae) against Anopheles gambiae (Diptera: Anophelidae) complex. Anim Res Int. 2017;6(3).
29. Bezerra JW, Costa AR, da Silva MA, Rocha MI, Boligon AA, da Rocha JB, et al. Chemical composition and toxicological evaluation of Hyptis suaveolens (L.) Poiteau (Lamiaceae) in Drosophila melanogaster and Artemia salina. S Afr J Bot. 2017;113:437-42.
30. Chukwujelewu JC, Smith P, Coombes PH, Mulholland DA, Van Staden J. Antiplasmodial diterpenoid from the leaves of Hyptis suaveolens. J Ethnopharmacol. 2005;102(2):295-7.
31. Anita S, Batish DR, Anu S, Singh HP, Gurpreet K. Chemical characterization and antioxidant activity of essential oil from the aerial parts of Hyptis suaveolens (L.) Poit. Int J Trop Agric. 2015;33(4(Part III)):3205-13.
32. Ghaffari H, Ghassam BJ, Nayaka SC, Kini KR, Prakash HS. Antioxidant and neuroprotective activities of Hyptis suaveolens (L.) poit against oxidative stress-induced neurotoxicity. Cell Mol Neurobiol. 2014;34(3):323-31.
33. Nayak P, Kar DM, Nayak S. In vitro [alpha]-Amylase Inhibition and Antioxidant potential of chloroform fraction of hydroalcoholic extract obtained from Hyptis Suaveolens. J Appl Pharm Sci. 2014;4(9): 46.
34. Poonkodi K, Karthika J, Tamilselvi V, Anitha R, Vasanthalmani S. Chemical composition of essential oil of Hyptis suaveolens (L.) poit and its in-vitro anticancer activity. J Pharm Res. 2017; 11(5):410-3.
35. Musa AK, Dike MC, Onu I. Evaluation of nitta (Hyptis suaveolens Poit.) seed and leaf extracts and seed powder for the control of Trogoderma granarium Everts (Coleoptera: Dermestidae) in stored groundnut. Am.-Eurasian J Agron. 2009; 2(3):176-9.
36. Sander LC. Soxhlet extractions. J Res Natl Inst Stand Technol. 2017;122:1.
37. Harborne JB. Methods of plant analysis. In; phytochem methods. Springer, Dordrecht. 1984;1-36.
38. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu
reagent. Methods Enzymol. 1999;299:152-78.

39. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995;28(1):25-30.

40. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. Anal. Biochem. 1996;239(1):70-6.

41. Jorgensen HJ. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. National Committee For Clinical Laboratory Standards Antimicrobial Susceptibility Testing. 1993:NCCLS-M7.

42. Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. Indian J Pharmacol. 2005;37(1):26-29.

43. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemoth. 2001;48(1):5-16.

44. Mishra AK, Mishra A, Tripathi S, Tripathi NN. Susceptibility of Enterococcus faecalis to the plant volatile oils. J. Microb. World. 2008;10:108-12.

45. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015;33(8):1582-614.

46. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res Fund Mol Mech Mut. 2005;579(1):200-13.

47. Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite profiling and wound-healing activity of Boerhavia diffusa leaf extracts using in vitro and in vivo models. J Tradit Complement Med; 2019.

48. Kumar V, Sharma N, Sourirajan A, Khosla PK, Dev K. Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of Terminalia arjuna from north-western Himalayas, India. J Tradit Complement Med. 2018;8(1):100-6.

49. Imlay JA, Chin SM, Linn S. Toxic DNA damage by hydrogen peroxide through the fenton reaction in vivo and in vitro. Science. 1988;240(4852):640-2.

50. Chandrasekara A, Daugelaite J, Shahidi F. DNA scission and LDL cholesterol oxidation inhibition and antioxidant activities of Bael (Aegle marmelos) flower extracts. J Tradit Complement Med. 2018;8(3):428-35.