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# Evaluation of Antioxidant Capacity and Safety Profile of Selected Medicinal Plant Extracts

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**Medicinal plants are widely recognised for their medicinal benefits, notably due to their bioactive phytochemicals and antioxidant capacity. The aim of the study is to investigate the phytochemical composition, antioxidant activity and cytotoxic effect of medicinal plants Ferula asafoetida Linn., Hyoscyamus niger Linn, Matricaria chamomilla Linn, Styrax benzoin Dryland., and Sesamum indicum Linn. Hydroalcoholic extracts of the medicinal plants were extracted using Soxhlet apparatus. Phytochemical screening was performed to identify the secondary metabolites. Quantitative analysis of protein, carbohydrates, total phenolic content (TPC) and flavonoids content (TFC). Antioxidant activity was evaluated using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the nitric oxide (NO) scavenging assay, Lipid peroxidation assay, ferric reducing antioxidant power assay and DNA damage protection against the oxidative stress induced by hydrogen peroxide. The toxicity evaluation is measured by the HET-CAM (Hen's Egg Test – Chorioallantoic Membrane) assay. The functional group detection is done by the FTIR (Fourier Transform Infrared) spectroscopy for better exposure to the identification of bioactive compounds. The results show that the phytochemical content of these plant extracts is dose dependent as the concentration of these extracts increases the phytochemical increase and eventually the antioxidant activity also increases. This notable behavior of these extracts aligns with the published data and suggests the efficiency of these plants in terms of therapeutics and health care.**

**Keywords:** Antioxidants; DNA damage; FTIR; Herbal medicine; HET-CAM; Phytochemicals.

The herbal plants were always known for its sources of some bioactive and derivative compounds that are essential for the antioxidant and eventually prevention of oxidative stress caused complications.[1] Among those plants, *Ferula asafoetida* resin, *Matricaria chamomilla* whole plant, *Sesamum indicum* seeds, *Styrax benzoin* resin, and *Hyoscyamus niger* seeds show maximum efficacy to the diseases. These plants are rich in different phytochemicals that

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target different metabolic and catabolic reactions, eventually balancing the reaction by neutralizing the by-products and some free radicals that are formed during the reaction. Traditionally these plants were used in different cultures for treating various health issues such as their ability to treat gastrointestinal complication, anti-inflammatory, sedative and to treat cold and flu. It is consumed as condiments, herbal tea and as oil. *F. asafoetida,* is well known for its ability to reduce the inflammation, diabetes and oxidative stress.[2,3] *H. niger* have unique photochemical systems that are essential for neutralizing the free radicals such as reactive oxygen species (ROS) and reduces oxidative stress.[4] *M. chamomilla is known* for its aroma used for calming, eventually by reducing the oxidative free radicals due to the presence of some unique flavonoids and steroids.[5] *S. indicum* and *S. benzoin* are enriched with terpenoids and phenolic compounds that are essential for antioxidant activity that promotes DNA repair and prevent DNA damage caused by oxidative stress and maintain the genomic stability.[6,7]

At the molecular level, these plants exhibit DNA repair phenomenon such as base excision repair and nucleotide excision repair, which is caused by oxidative stress to DNA. It improves and inhibits the immunological responses to the inflammation caused by the oxidate stress. Untreated, ignorance of having an unhealthy lifestyle can cause metabolic and catabolic imbalance caused by free radicals that increases oxidative stress, eventually triggers cell apoptosis precursors and also can lead to DNA mutation which can cause oncological disorder.[8] Hence to evaluate the toxicity effect of these medicinal plant Hen's Egg Test on Chorioallantoic Membrane (HET-CAM) assay is performed. The results estimate that the plant extracts were classified as non-irritant.

These findings suggest the plants exhibit favorable safety potential that enhance the potential for use in medicinal practices. The antioxidant and DNA repair potential of *F. asafoetida*, *H. niger, M. chamomilla, S. indicum*, and *S. benzoin* highlights their significance in protecting cellular integrity, offering the pathway to plant based therapeutic approaches.

## MATERIAL AND METHODS

**Sample preparation and authentication**

The extraction of plant samples was performed using Soxhlet extraction. In this process, 5 grams of powdered plant material were continuously extracted with a mixture of distilled water and methanol in a 7:3 v/v ratio. The extracts were then filtered and evaporated to dryness.[9] The plant materials were obtained from certified herbal medicine suppliers and verified by Dr. Rashid from Ajmal Khan Tibbia College, Aligarh Muslim University. Their taxonomic identification was officially verified with Certificate No. 843/DS.

**Qualitative analysis for phytochemicals**

Phytochemical detection involves various qualitative methods: carbohydrates are identified by Molisch's and Benedict's tests, amino acids by Ninhydrin, proteins by Biuret and Xanthoproteic tests, steroids by Salkowski and Liebermann-Burchard tests, glycosides by Borntrager's tests, flavonoids by Shinoda tests, phenols by lead acetate tests, tannins by Gelatin tests; terpenoids by Salkowski test; and alkaloids by Dragendorff's and Mayer's tests. These methods offer reliable preliminary screening for phytochemical analysis.[9,10]

**Quantitative analysis**

**Determination of Carbohydrate content**

The carbohydrate content was estimated by using the 3,5-dinitrosalicylic acid (DNSA) method, added 1ml of the plant extracts to 1ml of DNSA reagent. The reaction mixture is treated in boiling water bath for 300 seconds. Add 3ml of distilled water and optical density (OD) was measured at 525 nm.[11]

**Quantification of Protein content**

The hydroalcoholic extracts samples were mixed with Bradford reagent, which contains Coomassie Blue G-250 dye. Upon binding to protein, the dye undergoes a shift in optical density (OD), brown to blue color change is observed. The OD is recorded at 595 nm using a spectrophotometer. The protein concentration in the samples was then calculated based on the standard curve obtained from the BSA measurements.[12]

**Determination of phenolic content (TPC)**

The phenolic content of the hydroalcoholic plants extracts was evaluated using the Folin-

Ciocalteu (FC) method. Gallic acid is used as standard.[13] The extracts (1 mg/mL) was combined with 10% FC reagent (v/v). After a 5-minute incubation, 7.5% sodium carbonate (w/v) was added and allowed to react for 45 minutes at 37°C. The optical density (OD) was recorded at 765 nm using spectrophotometer against a blank. The results were represented as mg gallic acid equivalent per gram plants dry weight (mg GAE/g DW).[14]

**Total flavonoid content (TFC)**

The total flavonoid content of the plant extracts was assessed using the aluminum chloride colorimetric method with quercetin as the standard calibration curve.[15] The extracts (1 mg/mL) was mixed 10% aluminum chloride, 1 M potassium acetate, and of distilled water. After 30 minutes of incubation at 37°C, the optical density (OD) was recorded at 415 nm using a spectrophotometer against a blank. The results were represented as mg quercetin equivalent per gram of plants dry weight (mg QE/g DW).[14]

**Evaluation of antioxidant activity of extracts**

**Free radical scavenging assays**

To evaluate the extract's total antioxidant and free radical scavenging capacity, numerous assays are used, each based on a distinct mechanism.[16] The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay determines if the extract can donate hydrogen atoms or transfer electrons to neutralise DPPH radicals.[17] This technique produces a colour change from violet to yellow, which is measured at 517 nm with ascorbic acid as a calibration standard.[18] The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the scavenging activity of hydrophilic and lipophilic substances by lowering the ABTS•z radical cation.[19] For this experiment, the extract (concentrations ranging from 20 mg/ml to 100 mg/ml) is mixed with a pre-prepared ABTS solution, incubated for 30 minutes, and measured at 734 nm. A calibration curve is created using gallic acid.[16] The Nitric oxide (NO) scavenging assay evaluates the extract's capacity to neutralise nitric oxide radicals by preventing nitrite production [20]. This approach involves mixing the extract with freshly made Griess reagent, incubating it for two hours, and analysing it at 540 nm.[16] Each of these assays assesses the extract's antioxidant potential using spectrophotometric monitoring of colour changes.

$$Percent\ inhibition = \frac{Ao - As}{Ao} \times 100$$

Where Ao is the absorption of control and As is the absorption of the tested extract solution.

**Reducing antioxidant power assay (FRAP assay)**

The Ferric Reducing Antioxidant Power (FRAP) assay measures antioxidant activity by reducing ferric ions (Fe³z ) to ferrous ions (Fe²z ), resulting in a colour shift at 593 nm. In this test, 3 mL of FRAP reagent was mixed with 1 mL of plant extract (20-100 mg/mL) and incubated at 37°C for 30 minutes in the dark. The optical density (OD) was measured at 593 nm using Trolox as the standard, and samples were analysed in triplicate. The blank included all reagents except the sample.[21]

**Lipid peroxidation assay**

The lipid peroxidation (LPO) experiment involved combining 1 mL of plant extract with 1 mL of thiobarbituric acid (TBA). The mixture was then placed in a boiling water bath at 95°C for 60 minutes. After the test tubes had cooled to room temperature, the optical density (OD) was measured at 532 nm. To test linearity and MDA level in the plant extract, a standard calibration curve was created using malondialdehyde (MDA).[22]

**Investigation of $H_2O_2$-Mediated DNA Damage Using DNA Nicking Assay**

The DNA nicking assay evaluates the ability of substances to prevent or cause oxidative DNA damage by monitoring structural changes in plasmid DNA. In this study, pBR322 plasmid DNA (0.25 µg) was exposed to 60 mM $H_2O_2$ in 100 mM phosphate buffer (pH 7.4) at 37°C to induce damage. Hydroalcoholic extracts were tested for their protective effect across different conditions: DNA alone, DNA with $H_2O_2$, DNA with the extract, and DNA with both the extract and $H_2O_2$. Following incubation, the samples were analyzed using 1% agarose gel electrophoresis, and DNA bands were visualized with a Gel-Doc system to assess nicking and protection.[21]

**Irritation and toxicity assay: HET-CAM (Hen's Egg Test – Chorioallantoic Membrane) assay**

Fertilised hen eggs were procured from the Central Poultry Development Organisation

(Western Region), Aarey Milk Colony, Mumbai. They were incubated for 10 days at 37.8 ± 1.0°C and 45-65% humidity. On day ten, the eggs were candled to determine embryo viability, and any faulty eggs were eliminated. The shell of the air cell was removed, and the inner membrane was peeled back to reveal the vascularised Chorioallantoic membrane (CAM). A 0.2 mL sample of each test drug (n = 3) was placed on the CAM and left for 300 seconds. The test groups contained 0.1 M NaOH (positive control for haemorrhage and coagulation), 1% sodium lauryl sulphate (positive control for vasoconstriction), 0.9% NaCl (negative control), and hydroalcoholic extracts of *E. globulus*, *S. indicum*, *M. chamomilla*, and *F. asafoetida, H. niger, S. benzoin*. After 20 seconds, the CAM was rinsed with saline to observe vascular effects. The irritation index (RI) was calculated using the appropriate equation:

$$RI = \frac{301 - sH \times 5}{300} + \frac{301 - sL \times 7}{300} + \frac{301 - sC \times 9}{300}$$

where,

H = hemorrhage, C = coagulation, L = vascular lysis, RI = irritation index, and s = start second.[23] Based on their RI, the tested substances were categorized into three groups.

| Sr.no | Irritation category | Irritation index |
|---|---|---|
| 1. | Non-irritating | 0-0.9 |
| 2. | Irritating | 1-8.9 |
| 3. | Severe irritating | 9-21 |

**Fourier transform infrared spectroscopy studies**

The investigation was carried out utilising a Shimadzu FTIR spectrometer and the LabSolutions IR program. A little drop of hydroalcoholic plant extract was put to a dry NaCl window to produce a thin layer. The sample is subsequently placed into the sample holder, and the spectra are measured in the infrared band 4000-400 cm-1. The FTIR spectral peaks of plant extracts were analysed to discover functional groups linked with the bioactive substances contained in the phytochemicals of plant extracts.

**Table 1.** Phytochemical screening of Hydroalcoholic Extract

| Sr. No. | Phytochemical | *F. asafoetida* | *H. niger* | *M. chamomilla* | *S. indicum* | *S. benzoin* |
|---|---|---|---|---|---|---|
| 1. | Amino acids & Proteins | - | + | + | + | + |
| 2. | Carbohydrates | + | + | + | + | + |
| 3. | Steroids | - | - | + | - | - |
| 4. | Flavonoids | + | + | + | + | + |
| 5. | Glycosides | + | - | - | - | - |
| 6. | Tannins | - | - | - | - | - |
| 7. | Phenols | + | + | + | + | + |
| 8. | Alkaloids | - | - | + | + | - |
| 9. | Terpenoids | + | + | + | - | - |

**Table 2.** Extractive Yield, Phytochemical, Carbohydrate, and Protein Content of Medicinal Plants

| Sr. No. | Plant Name | Extractive Yield (%) ± SD | Total Carbohydrate Content ± SD (mg/ml) | Protein Content ± SD (mg/ml) | Phenolic Content ± SD (GAE/g) | Flavonoid Content ± SD (QE/mg) |
|---|---|---|---|---|---|---|
| 1 | *F. asafoetida* | 46.33 ± 0.34 | 0.45 ± 0.01 | 0.15 ± 0.01 | 0.29 ± 0.02 | 0.46 ± 0.03 |
| 2 | *H. niger* | 20.77 ± 0.08 | 0.27 ± 0.01 | 0.20 ± 0.01 | 2.93 ± 0.16 | 0.56 ± 0.02 |
| 3 | *M. chamomilla* | 27 ± 0.05 | 0.42 ± 0.00 | 0.97 ± 0.01 | 6.26 ± 0.11 | 0.35 ± 0.04 |
| 4 | *S. benzoin* | 19.77 ± 0.16 | 0.14 ± 0.01 | 0.14 ± 0.01 | 1.02 ± 0.05 | 0.41 ± 0.01 |
| 5 | *S. indicum* | 35 ± 0.87 | 0.14 ± 0.00 | 0.30 ± 0.05 | 1.13 ± 0.06 | 0.59 ± 0.02 |

## RESULTS

Extraction yields of hydroalcoholic extracts varied among the studied plants as shown in table 2. *F. asafoetida* showed the highest yield at 46.33%, while *S. benzoin* exhibited the lowest yield at 19.77%. *S. indicum*, *M. chamomilla*, and *H. niger* demonstrated moderate to low yields, with 35%, 27%, and 20.77%, respectively

### Qualitative analysis for phytochemical analysis

The phytochemical screening of the hydroalcoholic extracts revealed distinct compositions among the studied plants. Carbohydrates, flavonoids, and phenols were consistently present in all extracts as shown in table 1, while tannins were absent across the board. Amino acids and proteins were detected in *H. niger*, *M. chamomilla*, *S. indicum*, and *S. benzoin*, but not in *F. asafoetida*. Steroids were identified exclusively in *M. chamomilla*, while glycosides were detected solely in *F. asafoetida*. Terpenoids were found in *F. asafoetida*, *H. niger*, and *M. chamomilla*, and alkaloids were present in *M. chamomilla* and *S. indicum*.

### Quantitative analysis

### Total carbohydrate and protein content

Total carbohydrate content in the plant extracts varied significantly as shown in table 2. *F. asafoetida* exhibited the highest carbohydrate content at 0.45 mg/mL, followed by *M. chamomilla* with 0.42 mg/mL. *H. niger* showed a moderate carbohydrate content of 0.27 mg/mL, while *S. indicum* and *S. benzoin* had the lowest levels, both measuring 0.14 mg/mL. The protein content of the plant extracts showed considerable variation as shown in table 2. *M. chamomilla* exhibited the highest protein content at 0.97 mg/mL, while *S. benzoin* had the lowest at 0.14 mg/mL. Moderate protein levels were observed in *S. indicum* (0.30 mg/mL), *H. niger* (0.20 mg/mL), and *F. asafoetida* (0.15 mg/mL).

### Total phenolic and flavonoid content

The phenolic content of the plant extracts showed significant variation as shown in table 2. *M. chamomilla* exhibited the highest phenolic content at 6.26 ± 0.11 GAE/g, while *F. asafoetida* had the lowest at 0.29 ± 0.02 GAE/g. *H. niger* and *S. indicum* demonstrated moderate phenolic

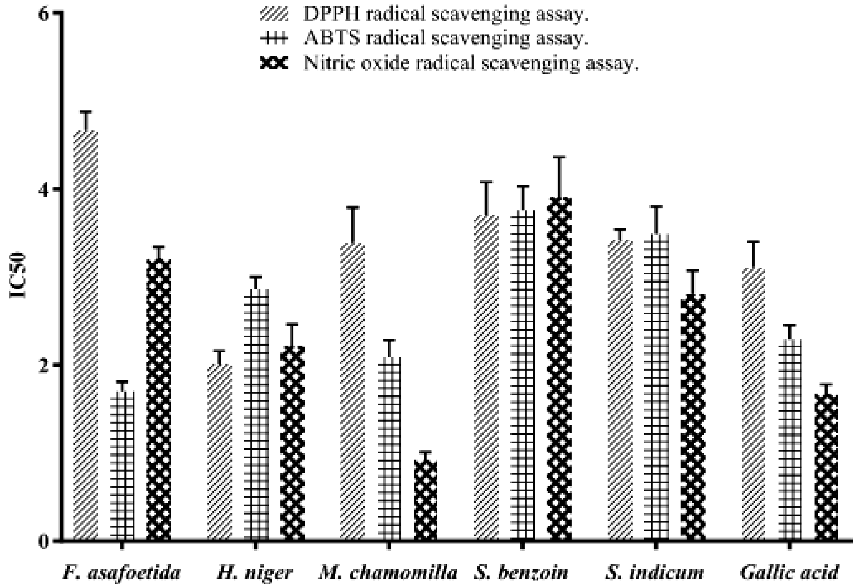


**Fig. 1.** The graph illustrates the IC… € (µg/mL) ± SD (n = 3) for DPPH, ABTS, and Nitric Oxide free radical scavenging assays, with statistical significance set at $p < 0.05$. The results indicate significant differences in antioxidant activity among the plant extracts, where lower IC… € values correspond to higher free radical scavenging potential

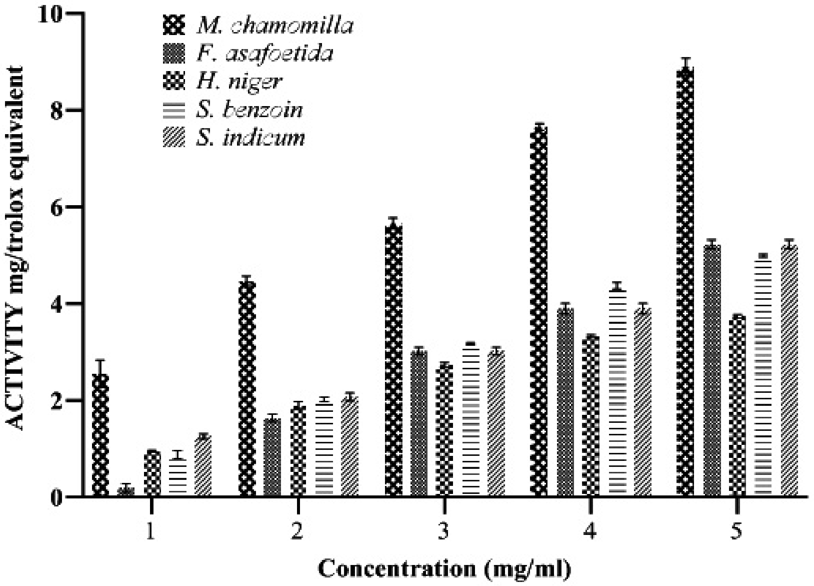


**Fig. 2.** Ferric Reducing Antioxidant Power (FRAP) of the hydroalcoholic extracts. n=3 with statistical significance set at $p < 0.05$

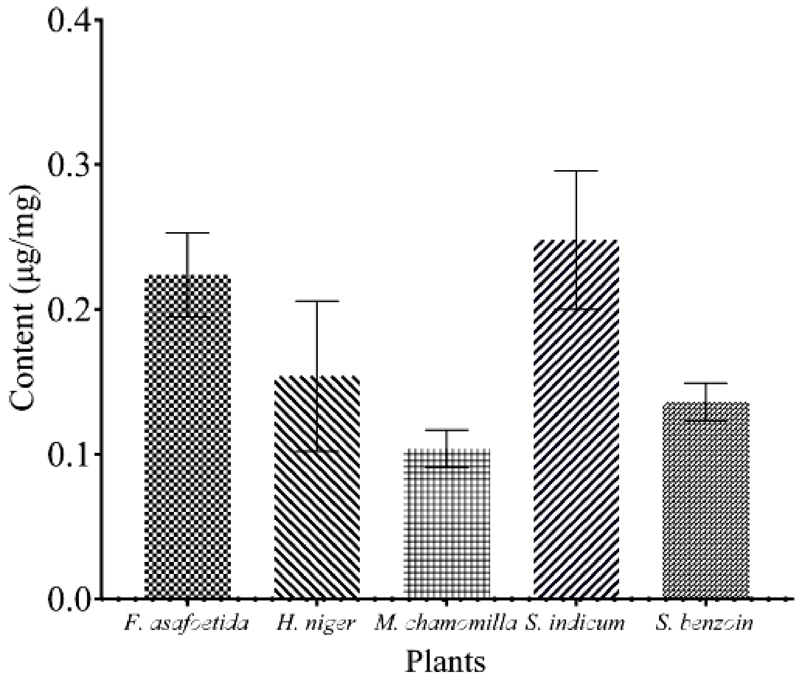


**Fig. 3.** The bar graph represents the MDA content (ig/mg extract) of hydroalcoholic extracts from different plant sources. The data is expressed as mean ± standard deviation (SD) for n = 3, and statistical significance was considered at $p < 0.05$

levels, measuring 2.93 ± 0.16 GAE/g and 1.13 ± 0.06 GAE/g, respectively. *S. benzoin* displayed a slightly lower phenolic content of 1.02 ± 0.05 GAE/g. The flavonoid content of the plant extracts varied across different species as shown in table 2, reflecting differences in their potential antioxidant activity. *S. indicum* exhibited the highest flavonoid content at 0.59 ± 0.02 QE/mg, followed closely by *H. niger* with 0.56 ± 0.02 QE/mg. *F. asafoetida* and *S. benzoin* showed moderate levels of flavonoids, measuring 0.46 ± 0.03 QE/mg and 0.41 ± 0.01 QE/mg, respectively. In contrast, *M. chamomilla* had the lowest flavonoid content at 0.35 ± 0.04 QE/mg.

The phytochemical content and antioxidant activity of hydroalcoholic extracts from *F. asafoetida*, *H. niger*, *M. chamomilla*, *S. benzoin*, and *S. indicum*. *M. chamomilla* exhibited the highest phenolic content at 6.26 ± 0.11 GAE/g and the highest total flavonoid content at 1.35 ± 0.04 mg quercetin equivalents as shown in table 2. In contrast, *F. asafoetida* had the lowest phenolic content at 0.29 ± 0.02 GAE/g but showed a relatively good ferric reducing antioxidant power (FRAP) activity, with values ranging from 0.19 to 5.23 µg/mL. All plant extracts demonstrated antioxidant potential across the DPPH, ABTS, FRAP, and nitric oxide (NO) scavenging assays, with IC… € varying among the plants, reflecting their differing levels of antioxidant efficiency.

The ABTS, DPPH, and nitric oxide (NO) IC50 values of the hydroalcoholic extracts showed significant variations in antioxidant activity. *H. niger* exhibited the lowest DPPH IC50 of 2.01 µg/mL, while *F. asafoetida* had the highest IC50 of 4.67 µg/mL. In the ABTS assay, *F. asafoetida* displayed the lowest IC50 of 1.7 µg/mL, whereas *S. benzoin* recorded the highest IC50 of 3.76 µg/mL, For NO scavenging, the IC50 values ranged

**Table 3.** Irritation scores and classification of plant extracts

| Sr. No. | Treatment | Irritation Score (RSD%) | Classification |
|---|---|---|---|
| 1 | 0.9% NaCl | 0.4 | Non irritating |
| 2 | 0.1 M NaOH | 18.77 | Extremely irritating |
| 3 | 1% sodium lauryl sulfate | 17.15 | Extremely irritating |
| 4 | *M. chamomilla* | 0.49 | Non irritating |
| 5 | *F. asafoetida* | 0.92 | Non irritating |
| 6 | *H. niger* | 0.54 | Non irritating |
| 7 | *S. benzoin* | 0.84 | Non irritating |
| 8 | *S. indicum* | 0.55 | Non irritating |

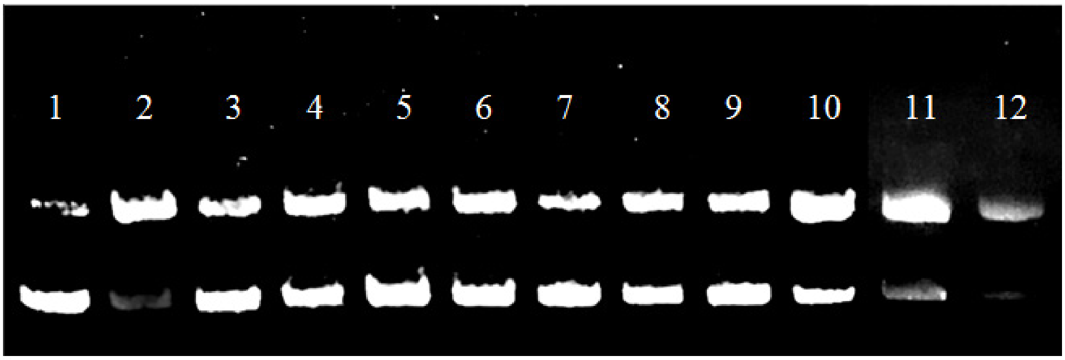


**Fig. 4.** Lane 1&2 - DNA & DNA+$H_2O_2$, Lane 3&4 - DNA+ *M. chamomilla* & DNA+ $H_2O_2$ + *M. chamomilla,* Lane 5&6 - DNA+ *F. asafoetida* & DNA+ $H_2O_2$+ *F. asafoetida,* Lane 7&8 - DNA+ *H. niger* & DNA+ $H_2O_2$+ *H. niger,* Lane 9&10 - DNA+ *S. benzoin* & DNA+ $H_2O_2$+ *S. benzoin,* Lane 11&12 - DNA+ *S. indicum* & DNA+ $H_2O_2$+ *S. indicum*

from 0.92 µg/mL to 3.91 µg/mL, demonstrating notable differences in nitric oxide neutralization efficiency. *M. chamomilla* showed the lowest IC50 of 0.92 µg/mL, while *S. benzoin* had the highest IC50 of 3.91 µg/mL. Lower IC50 values indicate higher antioxidant activity.

**Reducing antioxidant power assay (FRAP assay)**

FRAP (Ferric Reducing Antioxidant Power) activity of the hydroalcoholic extracts varied among the tested samples shown in figure 2, reflecting differences in their ferric-reducing potential. *M. chamomilla* exhibited the highest FRAP activity at 8.9 Trolox equivalent units, indicating its superior electron-donating ability. In contrast, *H. niger* showed the lowest activity at 3.74 Trolox equivalent units, suggesting comparatively weaker reducing power. The remaining extracts demonstrated moderate FRAP activity, with *S. indicum* at 5.23 Trolox equivalent units, *S. benzoin* at 4.99 Trolox equivalent units, and *F. asafoetida* at 3.9 Trolox equivalent units. Trolox, used as a reference standard.

**Lipid peroxidation assay**

The measured thiobarbituric acid-reactive substances (TBARS) in hydroalcoholic extracts showed interspecies variation, with *S. indicum* exhibiting the highest signal (0.248 ìg TBA-MDA equivalents/mg extract), followed by *F. asafoetida* (0.224 ìg/mg), *H. niger* (0.154 ìg/mg), *S. benzoin* (0.136 ìg/mg), and *M. chamomilla* (0.104 ìg/mg).

**Investigation of $H_2O_2$-Mediated DNA Damage Using DNA Nicking Assay**

The gel electrophoresis image demonstrates the effect of hydrogen peroxide (H, O, ) on DNA integrity and the potential protective role of various plant extracts as shown in figure 4. Lane 1 shows intact DNA, indicating no damage. In contrast, Lane 2 exhibits significant smearing, reflecting severe oxidative damage caused by H, O, . Lanes 3, 5, 7, 9, and 11 display well-defined bands, suggesting that *M. chamomilla*, *F. asafoetida*, *H. niger*, *S. benzoin*, and *S. indicum* do not degrade DNA under normal conditions.

The lanes containing DNA treated with both H, O, and plant extracts (Lanes 4, 6, 8, 10, and 12) show varying levels of protection against oxidative damage. Lanes 4, 6, 8, and 10 exhibit reduced smearing compared to Lane 2, indicating the antioxidant properties of the corresponding plant extracts, which likely mitigated the oxidative stress. However, Lane 12 shows persistent smearing, suggesting that the extract from *S. indicum* was not effective in preventing DNA damage caused by H, O, .

**Irritation and toxicity assay: HET-CAM (Hen's Egg Test – Chorioallantoic Membrane) assay**

The HET-CAM test is widely used as an alternative to in-vivo ocular irritation studies. It has lately been used as a preliminary screening tool to determine the irritating potential of compounds on membranes and skin prior to *in-vivo* testing.

The HET-CAM (Hen's Egg Chorioallantoic Membrane) assay was performed to evaluate the irritation potential of various plant extracts compared to standard irritants and a negative control. The results were expressed as the Irritation Score (IS). The standard irritants, 0.1 M NaOH and 1% sodium lauryl sulfate (SLS), had IS values of 18.77 and 17.15, respectively, categorizing them as extremely irritating. In contrast, the negative control, 0.9% NaCl, exhibited an IS of 0.4, indicating a non-irritating effect.

Among the plant extracts, *M. chamomilla* (IS = 0.49), *F. asafoetida* (IS = 0.92), *H. niger* (IS = 0.54), *S. benzoin* (IS = 0.84), and *S. indicum* (IS = 0.55) were all classified as non-irritating. The study was performed with a sample size of n = 3, and statistical analysis confirmed the significance of the results with a p-value $< 0.05$.

**Fourier transform infrared spectroscopy studies**

The various functional groups of the bioactive compound present in the plant extracts were analyzed by FTIR spectroscopy. Every spectral peak indicates the individual function groups and the intensity of the peaks indicates the quantity of that functional group containing compounds. The FTIR spectra of the hydroalcoholic extracts of *F. asafoetida*, *H. niger, M. chamomilla, S. indicum*, and *S. benzoin,* represents the existence of diverse functional groups in the extract which indicates the presence of various phytochemicals. The spectra show a common peak around 3400 $cm^{-1}$ indicates the presence of -OH stretching, confirms alcoholic and phenolic compounds, and peaks in the range 2920-2780 $cm^{-1}$ indicates the aliphatic and aromatic C-H stretching. A strong absorption peak is observed in the range of 1740- 1520 $cm^{-1}$ indicates the carbonyl (C=O), alkene (C=C), confirms the presence of esters, ketones and conjugated systems.

Peaks in 1250-1000 $cm^{-1}$ indicate (C-O) stretching associated with alcohol, esters and ethers. The peak between 700-600 $cm^{-1}$ confirms (C-X) halogen bonds, indicating halogenated compounds.

## DISCUSSION

Natural products have long been regarded as significant sources of bioactive chemicals with medicinal applications. In considering raising concerns about the adverse effects of synthetic medications, plant-based formulations provide safer and more sustainable alternatives. The hydroalcoholic extracts from *F. asafoetida*, *H. niger*, *M. chamomilla*, *S. benzoin*, and *S. indicum* exhibited considerable differences in extractive yields, reflecting their distinct phytochemical compositions. The notable yield from *F. asafoetida* can be attributed to its resinous nature, which shows high solubility in hydroalcoholic solvents. Phytochemical screening identified carbohydrates, flavonoids, and phenols in most extracts, indicating a shared antioxidant potential.[24] The presence of steroids in *M. chamomilla* suggests additional pharmacological applications, particularly in inflammation management. Likewise, the detection of glycosides in *F. asafoetida* supports its traditional use in treating oxidative stress and inflammation. These findings are consistent with previous reports highlighting the therapeutic properties of plant-derived bioactive compounds.[25,26]

A strong correlation between total phenolic, flavonoid content and the antioxidant activity was observed. Plants such as *M. chamomilla* and *H. niger* shows high TPC and TFC exhibits better antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Nitric Oxide (NO), Ferric Reducing Antioxidant Power (FRAP) and Lipid Peroxidation (LPO) assays. Phenolic compounds are well known for their electron donating ability, which leads to free radical neutralization. While flavonoids stabilize the reactive oxygen species (ROS). *F. asafoetida, S. indicum* and *S. benzoin* show moderate antioxidant activity but it increases with increase in the concentration this observation may be due to presence of distant bioactive cells that does not participate in the free radical scavenging and neutralizing the free radicals. The nitric oxide (NO) scavenging assay further confirmed the antioxidant capacity of the extracts, indicating their ability to neutralize reactive nitrogen species. Extracts with higher flavonoid and phenolic content, particularly *M. chamomilla*, showed superior NO scavenging effects, consistent with the reduction in oxidative stress biomarkers.[27,28]

Lipid peroxidation levels, determined by estimated malondialdehyde (MDA) content, gives additional interpretation into antioxidant potential. The MDA levels in *M. chamomilla* highlights strong antioxidant potential. It reduces lipid peroxidation and enhances protection against oxidative stress. *H. niger* and *S. benzoin* shows moderate MDA content indicates that it may be not as effective as *M. chamomilla* in lipid peroxidation. *F. asafoetida* and *S. indicum* show slightly elevated MDA content that highlights more lipid peroxidation may be due to the high concentration of extract, indicating greater oxidative stress and lower intrinsic antioxidant protection within these plant extracts.[27,29]

The DNA nicking assay evaluates the efficacy of the plant extracts in reducing and preventing oxidative DNA damage caused by $H_2O_2$ induced oxidative stress. The plant extracts neutralize the peroxidative damage to DNA by neutralizing the peroxide and due to this it reduces the DNA fragmentation. The extracts *M. chamomilla*, *F. asafoetida*, *H. niger*, and *S. benzoin* indicate notable antioxidant protection, possibly due to their polyphenol and flavonoid content.[30]

HET-CAM assay results indicate the toxicity of the plant extracts for topical applications including eyes. All plant extracts were classified as non-irritating, with *M. chamomilla* showing the lowest and *H. niger* and *S. indicium* almost show equal irritation score, supporting its potential use in skincare and pharmaceutical products. While the slightly higher irritation scores of *F. asafoetida* and *S. benzoin* may be due to their sulphur containing compounds or some other aromatic compounds like benzoic acid but still, they remained within the non-irritating range.

The FTIR spectrum indicates the presence of the functional groups that can indicate the presences of bioactive compounds like quercetin, ferulic acid, umbelliferon, scopolamine, benzoic acid and sesamol, these biomarkers are highlighted due their reported health benefit in medical sciences.

Notably, *the F. asafoetida* FTIR spectrum indicates phenolic compounds like ferulic acid and other compounds such as umbelliferon. *H. niger and M. chamomilla* show alkaloids like scopolamine and quercetin respectively. *S. indicum* shows phenolic lignans like sesamol. *S. benzoin* shows polyphenol like benzoic acid.

The hydroalcoholic extracts demonstrated various antioxidant and protective properties, these plants can be used in many therapeutics because of their superior antioxidant efficacy, high phenolic and flavonoid content, and safety profile. These results demonstrate and highlight that the plant base drugs are more efficient in terms of cost, efficacy and in low toxicity. Further research focusing on *in-vivo* efficacy, identification of biomarkers responsible for many therapeutic benefits, the isolation and characterization of specific bioactive compounds could enhance the understanding of their therapeutic mechanisms.

## CONCLUSION

The study evaluates the phytochemical composition, antioxidant potential and toxicity analysis of hydroalcoholic extracts of *F. asafoetida*, *H. niger*, *M. chamomilla*, *S. benzoin*, and *S. indicum*. *F. asafoetida* showed the highest extractive yield (46.33%), while *S. benzoin* had the lowest (19.77%). Phytochemical analysis confirms the presence of carbohydrates, proteins, flavonoids, and phenols. Antioxidant assays revealed significant activity, *H. niger* shows the highest DPPH scavenging (IC50 = 2.01 µg/mL), *M. chamomilla* show highest nitric oxide scavenging (IC50 = 0.92 µg/mL) and *F. asafoetida* shows highest ABTS scavenging activity (IC50 = 1.7 µg/mL). *M. chamomilla* showing the highest FRAP (8.9 Trolox equivalents) and lowest MDA content (0.104 ìg/mg extract), indicating lowest lipid peroxidation potential. The HET-CAM assay classified that the plant extracts as non-irritating, supporting their safety for topical and oral use. These results suggest their potential as a natural antioxidant for pharmaceutical and cosmetic applications. Further suggesting *in-vivo* studies to investigate and validate the therapeutic efficacy and toxicity. However, additional studies focusing on their mechanistic pathways, and *in-vivo* therapeutic efficacy will be crucial to unlocking their full potential in disease prevention and treatment.

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**Conflict of interest**

The authors do not have any conflict of interest.

**Data Availability Statement**

This statement does not apply to this article.

**Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials.

**Permission to reproduce material from other sources**

Not Applicable.

**Author Contributions**

Falak Bamne: Methodology, Investigation, Writing; Pawan Sharma: Data Curation, Formal Analysis, Validation, Writing; Ahmad Ali: Conceptualization, Supervision, Methodology, Resources; Munira Momin: Validation, Supervision; Tabassum Khan: Visualization, Data Presentation, Literature Review; Nikhat Shaikh: Methodology, Formal Analysis.

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