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RESEARCH ARTICLE

Antioxidant and antiglycation application of *Ferula asafoetida* resin and *Eucalyptus globulus* oil: A mechanism-based insight on diabetic research

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Abstract

The increasing dominance of diabetes and its complications has leads to search for natural antidiabetic and antiglycating compounds to control the harmful complications of advanced glycation end-products (AGEs). Natural products offer various therapeutic potentials over synthetic inhibitors, such as low toxicity and diverse biological activities. *Ferula asafoetida* Linn resin and *Eucalyptus globulus* Labill leaf oil were chosen based on traditional medical knowledge and scientific data demonstrating their bioactive components and pharmacological value in associated therapeutic applications. This study evaluates the therapeutic potential of *F. asafoetida* resin and *E. globulus* leaf oil as antiglycating co-relating phytochemical, antioxidant and antiglycation tests. The oleo-gum resin of *F. asafoetida* was extracted with distilled water using Soxhlet extraction, whereas *E. globulus* leaf oil was diluted using methanol in 1:10 ratio for analysis. The total phenolic and flavonoid contents was evaluated using Folin-Ciocalteu and aluminium chloride assays respectively and further GC-MS is used for phytochemical identification. The antioxidant activity was determined using (2,2-diphenyl-1-picrylhydrazyl) DPPH, (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS, nitric oxide scavenging tests and Ferri Reducing Antioxidant Power (FRAP). The antiglycation potential was determined by measuring fructosamine, protein carbonyl concentration, protein aggregation and AGEs formation. *E. globulus* oil exhibited higher phenolic and flavonoid content and antioxidant activity ($p < 0.05$), *while F. asafoetida* resin extract inhibited glycation intermediates and AGEs ($p < 0.05$). These findings point to their potential use in natural therapeutic formulations for diabetes-related oxidative and glycaemic stress.

Keywords: antioxidant; GC-MS; glycation; natural products; phytochemicals; protein aggregation

Introduction

Medicinal plants have played a vital role in human history, serving both as a source of nourishment and as a natural remedy for wide range of diseases and injuries. According to the data published by World Health Organization (WHO), approximately 80 % of the world population are dependent on plant base medicine, because of its cost efficiency, low toxicity and abundant in the nature. Apart from their traditional medicinal use, these plants are essential for modern drug development, by providing active pharmaceutical intermediates (API) (1). Multiple universally used drugs, such as morphine, vincristine, digoxin, digitoxin, reserpine, metformin and vinblastine, were isolated and derived from medicinal herbs and plants (2). These drugs were administered for the treatment of diabetes, cancer, hypertension, atherosclerosis, depression, asthma, obesity and other chronic complications. These ethnomedical plants demonstrate their persisting importance in both traditional therapeutic practices and modern medical research in drug delivery and discovery (3).

Ferula asafoetida Linn and *Eucalyptus globulus* Labill plants known for their significant and remarkable medicinal properties, especially in controlling diabetes and inflammatory precursors respectively. *F. asafoetida*, origin from places like Iran and Afghanistan, it is traditionally consumed as a vegetable and its oleoresin is used as a condiment also it is highly consumed to treat gastrointestinal disorders. This oleoresin is enriched with various phytochemicals like coumarins, sesquiterpenes and ferulic acid (4, 5). Ferulic acid, has various therapeutic properties including antioxidant that stabilize free radicals (Reactive oxygen species ROS, Reactive nitrogen species RNS etc.) eventually reducing oxidative stress and cytological damage. It is a significant and effective compound for the treatment of diabetes and other gastrointestinal complications (6, 7). *E. globulus*, originated from Tasmania, Australia and it is cultivated for its essential oil that is extracted from its leaves. The essential oil is known for its anti-inflammatory, antimicrobial and antioxidant properties due to the presence of a primary compound 1, 8-cineole (eucalyptol). Recent studies have demonstrated that 1, 8-cineole can reduce the oxidative stress and can regulates cytokines levels that leads to

inflammation related complications, factors that play a central role in managing diabetes and preventing complications associated with glycation (8, 9).

Although *F. asafoetida* and *E. globulus* are widely employed in herbal medicine and have been shown to have pharmacological effects (6, 8), there has been little study on their comparative antioxidant and anti-glycation activity under methylglyoxal (MG) induced glycation stress. The current study fills this gap by assessing the antioxidant and anti-glycation properties of *F. asafoetida* resin extract and *E. globulus* leaf oil. The antiglycation efficacy of these plants were evaluated using MG-induced glycation of bovine serum albumin (BSA) and gas chromatography-mass spectrometry (GC-MS) was used to identify the bioactive components responsible for their action. This approach facilitates the precise identification of volatile and semi-volatile compounds of these plant extracts. This study explores the therapeutic potential of these plants, especially their bioactive components, shows promising results to develop antiglycation and antioxidant drug delivery system to treat diabetes complications. The study also measured total phenolic and flavonoid content and antioxidant activity using DPPH, ABTS, nitric oxide scavenging and FRAP. The antiglycation activity was assessed by measuring fructosamine, protein carbonyl concentration, protein aggregation and Total AGEs formation. The observed decrease in AGEs, oxidative and carbonyl stress demonstrates the plants potential for creating innovative ways to glycation control and diabetes treatment. Based on these findings, standardised polyherbal formulations or topical treatments including *F. asafoetida* extract and *E. globulus* leaf oil may be developed to manage oxidative and glycation-related issues in diabetes patients.

Materials and Methods

Chemicals reagents and instruments

All chemicals and reagents used in the study were analytical grade and obtained from Sigma-Aldrich and Merck (Germany). Phytochemical test with Folin-Ciocalteu reagent, gallic acid, aluminium chloride and quercetin. Antioxidant tests with 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), sodium nitroprusside, sulfanilamide and naphthylethylenediamine dihydrochloride (NED). In antiglycation research, bovine serum albumin (BSA) and methylglyoxal (MG) were employed to induce glycation. Antiglycation indicators were evaluated using nitroblue tetrazolium (NBT) for fructosamine, 2,4-dinitrophenylhydrazine (DNPH) for protein carbonyl concentration and Congo red for protein aggregation. Shimadzu UV-1800 spectrophotometer was used to assess absorbance, while an Agilent spectrofluorometer was used for fluorescence.

Plant collection and identification

The plant samples were obtained from M/s Faquir Chand Pradeep Kumar, Kirana Kothi, Barahdwari, Aligarh and authenticated by Dr. Mohammad Rashid, Dept. of Saidla, Aligarh Muslim University. They were confirmed herbs *F. asafoetida* resin (Herbarium No: P-2022/S-14/AMU A) and *E. globulus* leaf oil (Herbarium No: P-2022/S-13/AMU), with Certificate No: 843. Soxhlet extraction was performed, where 5g of powdered resin was extracted with distilled water. After extraction, the water was evaporated at temperature 333 K and the dried extract was weighed to calculate the percentage yield (10). The resulting extract was stored at 274 K for further use. *E. globulus* oil was diluted in 100 % methanol (1:10) and then used for biochemical assays.

$$\text{Percentage Yield: } \frac{\text{Weight of Powdered Material}}{\text{Weight of Extract}} \times 100$$

Phytochemical screening

The resin extracts and oil were treated with various standard reagents for phytochemical analysis, including alkaloids, carbohydrates, glycosides, phenolics, flavonoids, tannins, proteins and steroids, using standard qualitative methods. Alkaloids were detected with Dragendorff's reagent, confirms with orange or reddish-brown precipitate. Carbohydrates were analyzed through Molisch test, indicated by the formation of purple or violet ring. Glycosides were identified using the Keller-Killani test, formation of reddish-brown ring at the interface. Phenolics were analyzed with ferric chloride test, resulting in a characteristic blue, green, or black coloration. Flavonoids were detected by the addition of lead acetate, forming a yellow precipitate. Tannins were confirmed using Braymer's test, producing a green or blue-black coloration. Proteins were identified through the Biuret test, confirmed by the development of a violet color. Steroids were analyzed using the Liebermann-Burchard Test, which formed a green or bluish green colored complex (11, 12).

Total Phenolic Content (TPC)

The TPC was estimated by the Folin-Ciocalteu method with standard gallic acid (Fig. 1A). The plant extracts were mixed with Folin-Ciocalteu reagent and sodium carbonate. After 45 min, absorbance at 765 nm was measured. Results were calculated using slope point formula and represented as mg per gram as gallic acid equivalent (mg GAE/g) (13, 14).

Total Flavonoid Content (TFC)

The TFC was estimated by the Aluminum chloride ($AlCl_3$) method with standard Quercetin (Fig. 1B). Plant extract was mixed with methanol, $AlCl_3$, potassium acetate and distilled water. After 30 min, measured absorbance at 415 nm. Results were calculated using slope point formula and represented as mg per gram as quercetin equivalent (mg QE/g) (15, 16).

Free radical scavenging assay

To evaluate the free radical scavenging potential of the plant extract, following antioxidant assays were employed based on different mechanisms The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay measures antioxidant ability to donate hydrogen or transfer electrons to neutralize DPPH radicals, causing a color change from violet to yellow, measured at 517 nm for calibration ascorbic acid (Fig. 2A) is used. The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay evaluates the scavenging of both hydrophilic and lipophilic species, with the reduction of the $ABTS^{\bullet+}$ radical cation. The extract (ranging from 20 mg/mL to 100 mg/mL) were mixed with a pre-prepared solution of ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), followed by a 30 min incubation, monitored at 734 nm for calibration curve gallic acid is used. The NO (Nitric Oxide) scavenging assay assesses the extract's ability to neutralize nitric oxide radicals by inhibiting nitrite formation. The extract was mixed with freshly prepared Griess reagent followed by 2 hr

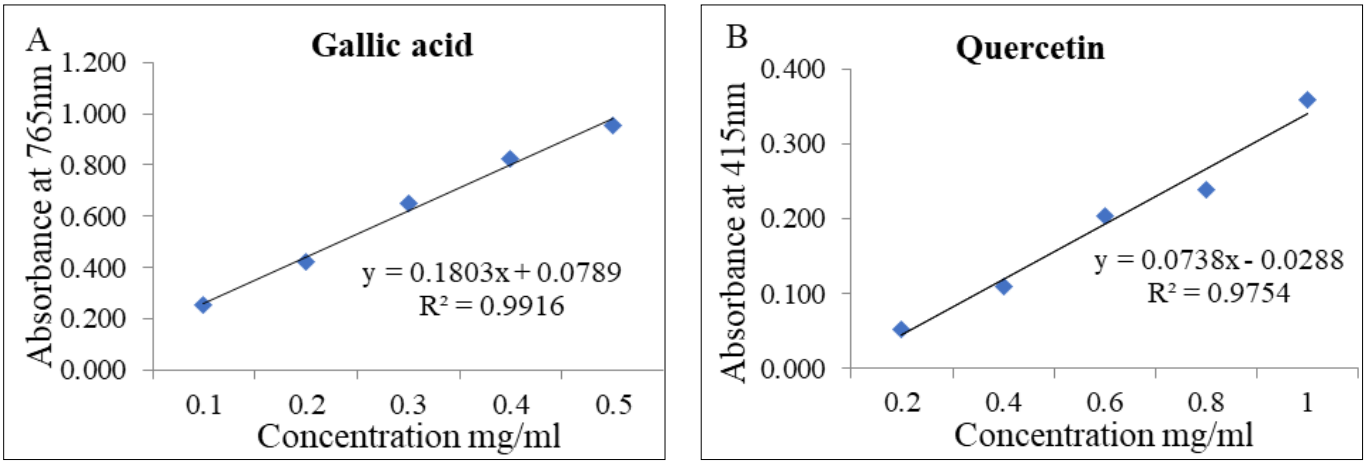

Fig. 1. Standard calibration curve. A: Total phenolic content (Gallic acid). B: Total flavonoid content (Quercetin).

incubation and monitored at 540 nm. Each method quantifies the extract's antioxidant capacity through spectrophotometric analysis of colour changes (17-22).

Percentage inhibition:

$$\frac{\text{Absorbance of control - Absorbance of Extract}}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the antioxidant capacity of the plant extract by its potential to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. The FRAP reagent is prepared by mixing acetate buffer (pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) solution and ferric chloride solution in a 10:1:1 ratio. The plan extracts were treated with the reagent and incubated at 37 °C for 4-6 min, after forming a blue-coloured ferrous-TPTZ complex, the absorbance is measured at 593 nm. Trolox is used as standard to prepare a calibration curve (Fig. 2C) and the antioxidant activity of the sample is expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (17, 23).

In-vitro antiglycation studies

The antiglycation reaction mixes were created to determine the inhibitory effects of *F. asafoetida* resin extract and *E. globulus* leaf oil on methylglyoxal (MG)-induced protein glycation. The experimental setting includes five groups, with bovine serum albumin (BSA) serving as the model protein a control group containing BSA alone, negative control group containing BSA

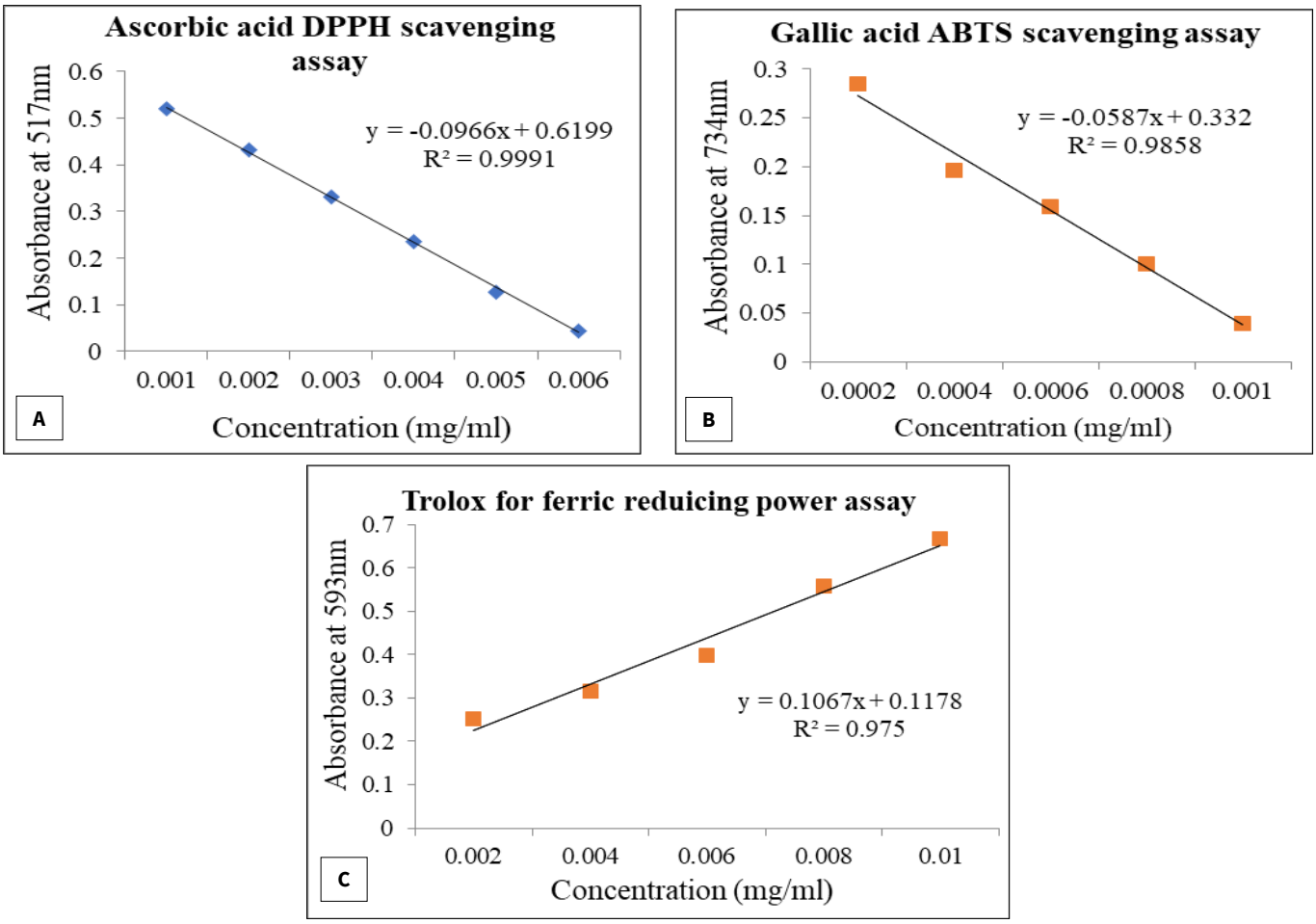

Fig.2. Standard calibration curve. A: Ascorbic acid for DPPH assay, B: Gallic acid for ABTS radical scavenging assay and C: Trolox for FRAP assay.

and MG, test group-1 containing BSA and *F. asafoetida* resin extract and BSA and *E. globulus* oil, MG-induced test groups containing BSA, MG and either *F. asafoetida* extract and *E. globulus* oil. To prevent microbiological contamination, all reaction mixtures were prepared in 100 mM phosphate buffer (pH 7.4) with 3 mM sodium azide. They were then incubated at 37 °C for five days in the dark. This approach enabled the investigation of both the individual and combined effects of the resin extract and essential oil in preventing or inhibiting glycation-related protein changes (14).

Analysis of fructosamine content by NBT (nitroblue tetrazolium) assay

The NBT assay was used to determine the fructosamine content. The glycated sample was mixed with NBT solution in carbonate buffer (pH 10.4) and incubated for 15 min at 37 °C and absorbance was measured at 530 nm. 1-deoxy-1-morpholino-fructose is use as standard and for calculating fructosamine content (16).

Analysis of protein aggregation by Congo red assay

For the Congo red assay was used to estimate the formation of protein aggregates, the mixture of Congo red dye and glycated samples was incubated for 20 min. The absorbance was measured at 530 nm (14).

Analysis of carbonyl content by DNPH assay

For carbonyl content estimation, the glycated sample was treated with DNPH in HCl and incubated for 1 hr at 37 °C. Then, trichloro-acetate was added, followed by centrifugation. The pellet was washed with ethanol: ethyl acetate in 1:1 ratio, the pallets were dissolved in guanidine HCl and absorbance was measured at 370 nm (3). Carbonyl content was calculated (24).

Analysis of total (advanced glycation end products) AGEs by spectrofluorometer

To measure fluorescence of AGEs formed during bovine albumin glycation, samples were prepared by diluting incubated sample with distilled water in a ratio 1:10. Measurements were conducted using an Agilent Cary Eclipse spectrofluorometer with excitation at 370 nm and emission at 440 nm (14, 16).

GC-MS analysis of *F. asafoetida* resin extract and *E. globulus* leaf oil

The chemical composition of *F. asafoetida*, along with *E. globulus*, was analyzed using GC-MS (Shimadzu GCMS-QP2010 Ultra) equipped with an RTX-5 capillary column (30 m × 0.25 mm × 0.25 µm). Helium (1 mL/min) was used as the carrier gas, with a split ratio of 1:50 and 1 µL of the sample (10 % in hexane) was injected. The GC oven temperature was programmed from 60 °C to 210 °C (3 °C/min), then to 240 °C (20 °C/min, held for 8.5 min). The injector and detector temperatures were set at 280 °C and 290 °C, respectively. The mass spectrometer (dual-stage TMP ultra system) operated at 70 eV, scanning 40-550 amu. Compounds were identified using the Adams table and NIST MS Library, providing a detailed chemical profile of the extracts (25, 26).

Statistical analysis

Statistical analysis was performed using GraphPad Prism and Microsoft Excel. Data are expressed as the mean ± standard error of the mean (SEM) from three independent experiments (n = 3). A P value < 0.05 was considered statistically significant.

Results

Phytochemical screening

The qualitative phytochemical analysis of the aqueous extracts of *F. asafoetida* and *E. globulus* revealed distinct profiles (Table 1) for both plants. *F. asafoetida* tested positive for carbohydrates, amino acids and proteins, glycosides, flavonoids, phenols, steroid, tannins, terpenoids and alkaloids, indicating a rich presence of water-soluble bioactive compounds. *E. globulus* exhibited positive results for steroids, glycosides, flavonoids, phenols, tannins, terpenoids and alkaloids, confirming the presence of key phytochemicals that contribute to its potent antioxidant and medicinal properties. This phytochemical screening highlights the diverse array of bioactive compounds in both plants with *E. globulus* showing a particularly strong presence of phenolics and flavonoids.

Total phenolic and total flavonoid content

The percent yield of extract is 41.39 ± 0.29 obtained from *F. asafoetida* procured by Soxhlet extraction method with distilled water as a solvent. *E. globulus*, concentrated essential oil was employed and it is diluted with methanol to prepare a 10 % working solution. *F. asafoetida* exhibited a total phenolic content of 0.19 ± 0.01 mg GAE/g and a flavonoid content of 0.50 ± 0.12 mg QE/g. *E. globulus* showed significantly higher values, with a total phenolic content of 2.45 ± 0.59 mg GAE/g and a flavonoid content of 4.84 ± 0.079 mg QE/g.

Free radical scavenging assay

The IC50 values for *F. asafoetida* in the DPPH, ABTS and NO scavenging assays indicate a moderate level of antioxidant activity (Fig. 3A). The NO assay exhibits the lowest IC50 value (2.96 mg/mL), suggesting better efficacy in neutralizing nitric oxide radicals compared to DPPH (4.44 mg/mL) and ABTS (3.24 mg/mL). However, the relatively higher IC50 values suggest that *F. asafoetida* requires a higher concentration to achieve 50 % inhibition *E. globulus* demonstrates significantly lower IC50 values across all assays (Fig. 3B), with an exceptionally low IC50 of 0.027 mg/mL in the ABTS assay, highlighting its strong antioxidant potential. The DPPH (1.011 mg/mL) and NO (1.457 mg/mL) assays also exhibit lower IC50 values compared to *F. asafoetida*, suggesting superior antioxidant activity.

Table 1. Phytochemical analysis of aqueous plant extracts of *F. asafoetida* and *E. globulus*

Sr. No	Phytochemical	*F. asafoetida*	*E. globulus*
1	Carbohydrate	+	+
2	Amino acids/ Proteins	+	-
3	Steroids	+	+
4	Glycosides	+	+
5	Flavonoids	+	+
6	Phenols	+	+
7	Terpenoids	+	+
8	Tannins	+	+
9	Alkaloids	+	+

This experiment was performed in triplicates (n = 3) and the results were statistically analyzed, with a p-value < 0.05 considered significant.

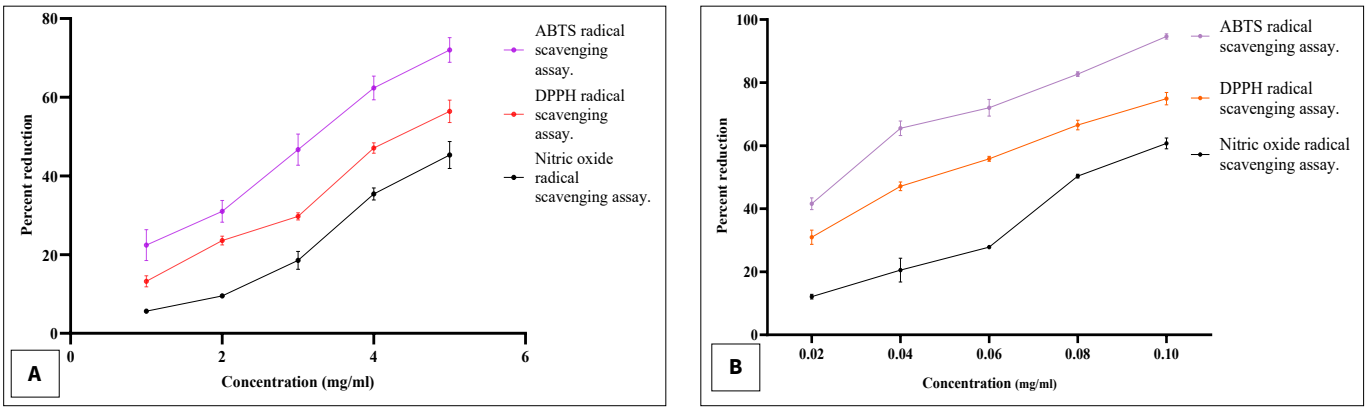

Fig. 3. Free radical scavenging activity of *F. asafoetida* (A) and *E. globulus* oil (B) measured by ABTS, DPPH and NO assay. This experiment is performed in triplicates n=3 and p-value<0.05.

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant activity, as assessed by the FRAP assay, increases with rising concentrations of the extracts. *E. globulus* exhibits the highest antioxidant potential (Fig. 4B), while *F. asafoetida* demonstrates the lowest (Fig. 4A). All extracts display an antioxidant effect comparable to that of Trolox (Fig. 2C).

Analysis of fructosamine content by NBT (nitroblue tetrazolium) assay

Methylglyoxal effectively induces protein glycation, as shown by the increased fructosamine content in BSA+MG (Fig. 5). However, both *F. asafoetida* and *E. globulus* reduce fructosamine levels, likely due to their antioxidant properties counteracting oxidative stress. *F. asafoetida* shows slightly greater antiglycation potential than *E. globulus*.

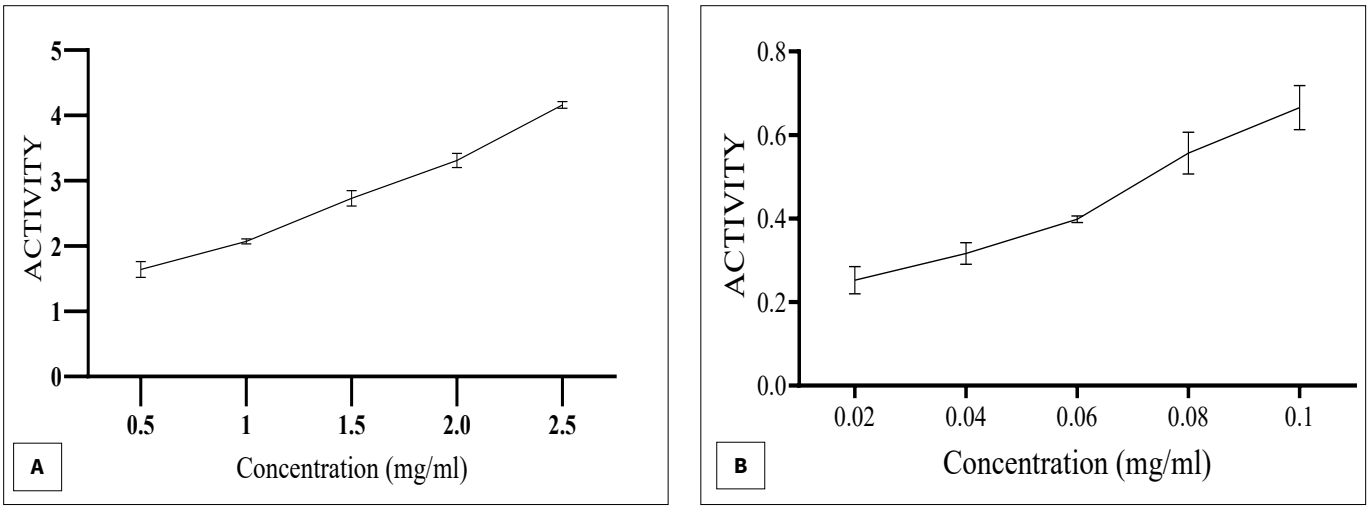

Fig. 4. Ferric reducing power of *F. asafoetida* (A) and *E. globulus* oil (B). This experiment is performed in triplicates n=3 and p-value<0.05.

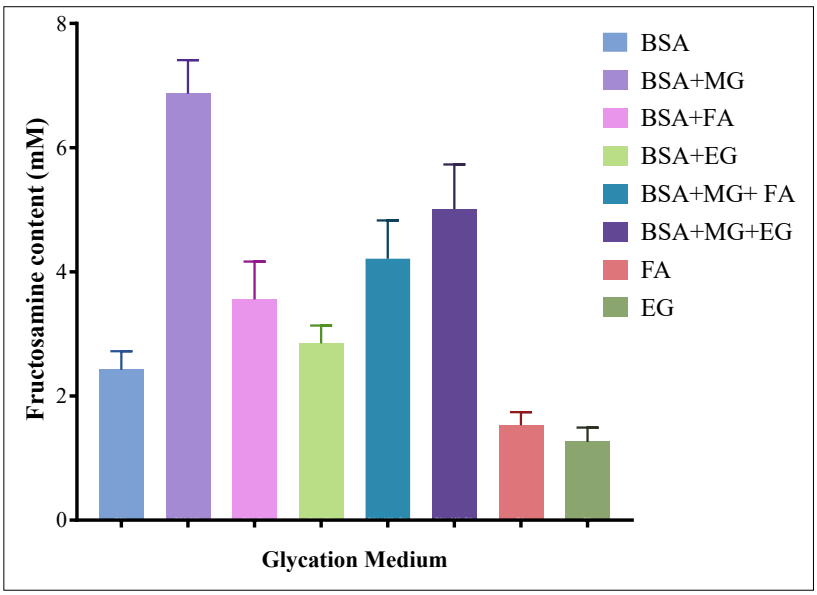

Fig. 5. Fructosamine content in bovine serum albumin (BSA) After 5-day incubation using a methylglyoxal (MG)-induced glycation model. Fructosamine levels were measured to evaluate the antiglycation potential of *F. asafoetida* (FA) resin and *E. globulus* (EG). This experiment is performed in triplicates n=3 and p-value<0.05.

Analysis of protein aggregation by Congo red assay

In the Congo Red assay, BSA+MG shows increased absorbance (Fig. 6), indicating significant protein aggregation due to methylglyoxal-induced glycation. *F. asafoetida* and *E. globulus* do not promote aggregation, as seen in BSA+FA and BSA+EG. Lower absorbance in BSA+MG+FA and BSA+MG+EG suggests their ability to prevent aggregation, likely by scavenging carbonyl species or enhancing antioxidant defenses.

Analysis of carbonyl content by DNPH assay

Methylglyoxal significantly increases carbonyl content (Fig. 7), indicating strong glycation or oxidative stress. *F. asafoetida* and *E. globulus* reduce this effect in BSA+MG+FA and BSA+MG+EG, with *F. asafoetida* being more effective. The extracts do not promote carbonyl formation (BSA+FA, BSA+EG).

Total AGEs by spectrofluorometry

The graph represents the formation of Advanced Glycation End Products in Bovine Serum Albumin. The sample treated with methylglyoxal shows the highest absorbance peak, indicating a significant increase in AGEs formation, which is expected due to methylglyoxal's role as a potent glycating agent (Fig. 8).

BSA treated with methylglyoxal and *F. asafoetida* or *E. globulus* shows reduced absorbance, suggesting inhibition of AGEs formation due to their antioxidant properties. BSA+FA and BSA+EG without methylglyoxal exhibit minimal AGEs, reinforcing their protective effects. The control BSA sample shows almost no AGEs, as expected. *F. asafoetida* and *E. globulus* alone do not contribute to glycation, supporting their role as protective agents. Overall, both extracts help reduce AGEs formation, particularly in the presence of methylglyoxal.

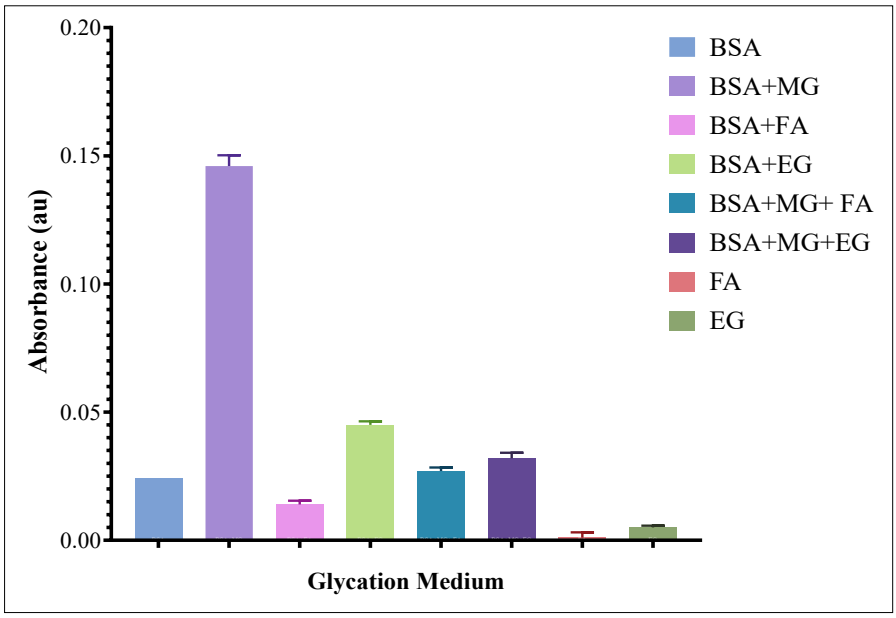

Fig. 6. Formation of protein aggregates in bovine serum albumin (BSA) After 5-day incubation using a methylglyoxal (MG)-induced glycation model. Protein aggregation levels were assessed to determine the antiglycation effects of *F. asafoetida* (FA) resin and *E. globulus* (EG). This experiment is performed in triplicates n=3 and p-value<0.05.

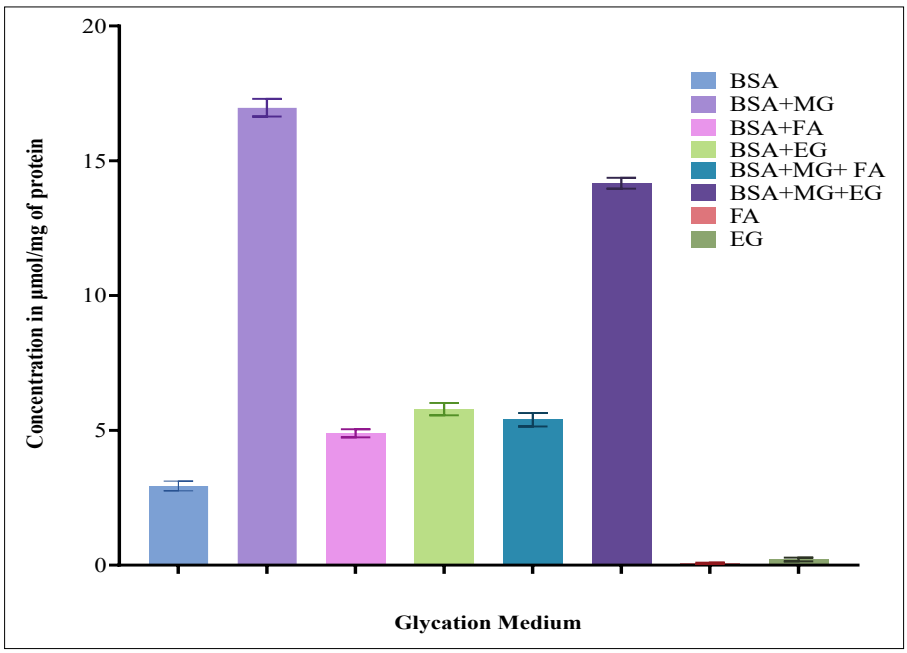

Fig. 7. Concentration of carbonyl formed in the glycation medium after 5-day incubation using a methylglyoxal (MG)-induced glycation model with bovine serum albumin (BSA). The carbonyl content was measured to evaluate the antiglycation potential of *F. asafoetida* (FA) resin and *E. globulus* (EG). This experiment is performed in triplicates n=3 and p-value<0.05.

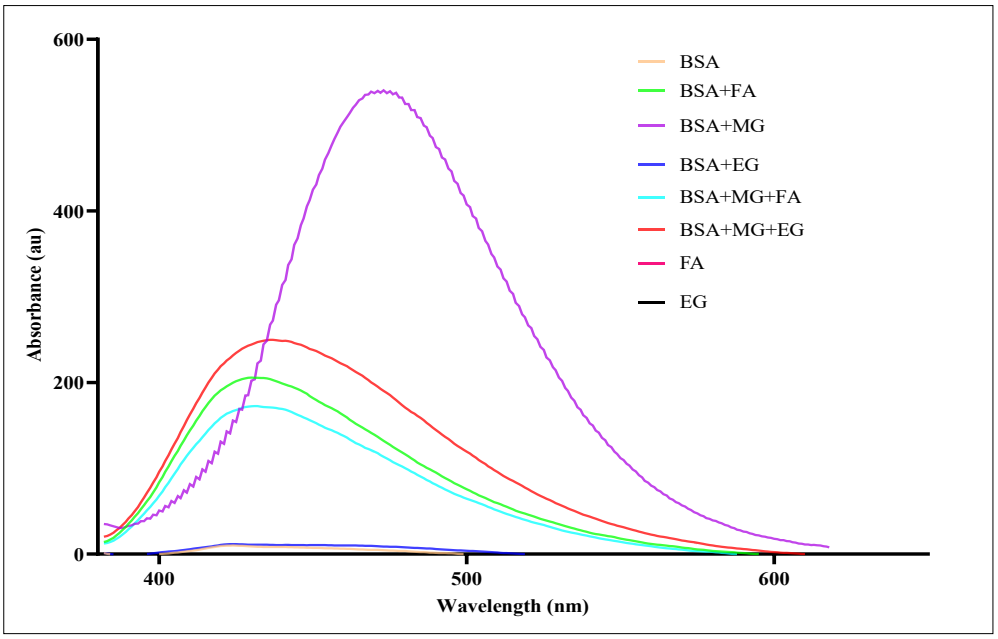

Fig. 8. Formation of total advanced glycation end-products (AGEs) in the glycation medium. After 5-day incubation using a methylglyoxal (MG)-induced glycation model with bovine serum albumin (BSA). Total AGEs levels were measured to assess the antiglycation effects of *F. asafoetida* (FA) resin and *E. globulus* (EG). This experiment is performed in triplicates n=3 and p-value<0.05.

GC-MS analysis

The GC-MS analysis of *F. asafoetida* and *E. globulus* shown in Table 2 extracts identified various bioactive compounds, predominantly terpenoids, fatty acids, aldehydes and sulfur-containing compounds. *E. globulus* exhibited a high concentration of oxygenated monoterpenes, including eucalyptol (1,8-cineole), α-terpineol and D-limonene, as well as sesquiterpenes like (-)-globulol, viridiflorol and γ-eudesmol (Table 2). Additionally, it contained monoterpene hydrocarbons, such as camphene, β-myrcene and γ-terpinene.

F. asafoetida was rich in fatty acids, including myristic acid, palmitic acid, linoleic acid and stearic acid, along with aldehydes like oleic aldehyde (9-octadecenal). The extract also contained sulfur-based compounds (Table 2), such as bis (methylthio) propane and dithiolane, which contribute to its strong characteristic odour.

Discussion

The current study evaluates the antioxidant and antiglycation effects of *F. asafoetida* oleoresin and *E. globulus* essential oil using phytochemical profiling and mechanism-based bioassays. The oleoresin of *F. asafoetida* was extracted using the Soxhlet apparatus and the essential oil of *E. globulus* was applied directly without additional processing. These changes in extraction procedures and sample composition had a considerable impact on their phenolic (TPC), flavonoid (TFC) and biological activity. At

Table 2. Major chemical components identified in *Ferula asafoetida* and *Eucalyptus globulus*

Sr. No.	Chemical Compound	Class	RT (min)	Source (*F. asafoetida* / *E. globulus*)
1	Eucalyptol (1,8-cineole)	Oxygenated monoterpene	7.950-8.322	*E. globulus*
2	D-Limonene	Monoterpene hydrocarbon	7.373, 8.285	*E. globulus*
3	α-Terpineol	Oxygenated monoterpene	11.512, 12.329	*E. globulus*
4	γ-Terpinene	Monoterpene hydrocarbon	8.524	*E. globulus*
5	(-)-Globulol	Sesquiterpenoid	22.542	*E. globulus*
6	Viridiflorol	Sesquiterpenoid	22.704	*E. globulus*
7	Alloaromadendrene	Sesquiterpene	19.314	*E. globulus*
8	Geranyl acetate	Monoterpene ester	17.404	*E. globulus*
9	Myristic acid (Tetradecanoic acid)	Fatty acid	23.967	*F. asafoetida*
10	Palmitic acid (n-Hexadecanoic acid)	Fatty acid	28.097, 31.001	*F. asafoetida* / *E. globulus*
11	Linoleic acid (9,12-Octadecadienoic acid)	Polyunsaturated	32.598	*F. asafoetida*
12	Stearic acid (Octadecanoic acid)	Fatty acid	31.705	*F. asafoetida*
13	Oleic aldehyde	Aldehyde	28.449	*F. asafoetida*
14	Bis(methylthio)propane	Sulfur-containing compound	15.442-15.692	*F. asafoetida*
15	Dithiolane	Heterocyclic-sulfur compound	7.592-8.838	*F. asafoetida*
16	Camphene	Monoterpene	5.357	*E. globulus*
17	β-Myrcene	Monoterpene	6.415	*E. globulus*
18	α-Guaiene	Sesquiterpene	19.995	*E. globulus*
19	γ-Eudesmol	Sesquiterpene alcohol	23.632	*E. globulus*
20	8-epi-γ-Eudesmol	Sesquiterpenoid	20.479	*F. asafoetida*
21	3-Carene	Monoterpene	6.849	*E. globulus*

the phytochemical level, *F. asafoetida* showed low TPC and TFC at lower doses, although both rose concentration-dependently. In contrast, *E. globulus* oil exhibited relatively high TPC and TFC levels even at low concentrations, with comparable increases at higher dosages. This shows that the essential oil has a greater initial phytoconstituent profile than the resin extract.

In terms of antioxidant activity, *F. asafoetida* demonstrated poor radical scavenging at low doses but considerably enhanced activity as concentrations climbed. These findings are consistent with previous research that has found moderate antioxidant capabilities in *Ferula* species, mostly due to their phenolic and flavonoid components (24, 25). Meanwhile, *E. globulus* exhibited considerable antioxidant activity at all doses examined, validating earlier research that relates this potency to its high levels of eucalyptol, rutin, flavonoids and terpenes. These compounds are well-known for their potential to lower oxidative stress while also providing neuroprotective and anti-inflammatory potential (26-28).

Antiglycation tests demonstrated a more noticeable difference between the two species. Even at doses utilised in antioxidant experiments, *F. asafoetida* had a substantial antiglycation capability. The superior action is most likely caused by its sulfur-containing compounds, which can trap or stabilise reactive nitrogen species (RNS), reactive oxygen species (ROS) and, in particular, reactive carbonyl species (RCS), which are glycation intermediates. These findings are congruent with the literature, which describes sesquiterpene coumarins and sulphurous metabolites from *Ferula* species as effective antiglycation agents that protect against protein glycation and other diabetes problems (29,30). In contrast, *E. globulus* oil had only mild antiglycation benefits, which were most noticeable at higher dosages. While it inhibited the synthesis of fructosamine and protein carbonyls, it was less effective than *F. asafoetida* at lowering protein aggregation and overall AGEs formation (31). These findings back up previous research describing *E. globulus* as a potent antioxidant and anti-inflammatory agent with mild to moderate antiglycation activity. It is likely that *E. globulus* anti-inflammatory capabilities indirectly diminish glycation-related damage by reducing oxidative stress, which increases carbonyl stress in diabetes (32). The antioxidant activities of both plants are linked to the neutralisation of free radicals via polyphenols, flavonoids and alcohols in *E. globulus* and to a lesser extent, sulfur-containing substances in *F. asafoetida*. In contrast, antiglycation actions appear to be more dependent on the plant capacity to trap reactive carbonyl species like glyoxal or methylglyoxal, which contribute to early glycation products like fructosamine and later-stage AGEs. Certain phytochemicals, such as resveratrol, quercetin, genistein and hesperidin derivatives, have been shown to stabilise these intermediates and similar effects are hypothesised for *F. asafoetida* sulfur-rich fractions (33-36).

The study also found a concentration-dependent relationship between antioxidant and anti-glycation activities. As antioxidant levels increases, the inhibition of glycation markers like fructosamine and carbonyl content improves, suggesting that oxidative stress neutralization may help to reduce glycation damage. This lends support to the hypothesis that there is a relationship between oxidative burden and glycation intensity (37). When compared to previous investigations, the new findings corroborate *E. globulus* high antioxidant profile, which is compatible with its identified phytoconstituents and health advantages. In contrast, while *F. asafoetida* is often stated to have low antioxidant capacity, this work highlights its powerful antiglycation action, which is due to components that are usually overlooked in normal polyphenol-based analysis. Both plants have previously been linked to antidiabetic, anti-inflammatory and metabolic regulating effects, which are supported by the biochemical data mentioned above. Importantly, this study provides considerable comparative information by directly evaluating *F. asafoetida* resin and *E. globulus* leaf oil under identical test circumstances. This method reveals each plant individual functional strengths: *F. asafoetida* excels at antiglycation, whilst *E. globulus* is an excellent antioxidant. This distinction is critical for creating tailored phytotherapeutic formulations, especially for illnesses such as diabetes, where both oxidative and glycaemic stress contribute to disease development. The study's practical benefits emerge from its prospective use. *F. asafoetida* may be an effective remedy for treating glycation-related problems such diabetic neuropathy and neurodegenerative disorders (29). Meanwhile, *E. globulus* oil, with its potent antioxidant and anti-inflammatory characteristics, may be more suited for formulations that aim to reduce oxidative stress, inflammation and tissue damage (32). These findings establish a mechanistic framework for future product development in both nutraceutical and pharmaceutical situations.

Furthermore, the research highlights the necessity of further *in vivo* validation to validate the reported effects under physiological circumstances. It also recommends conducting dissolution tests at various pH levels to assess the release and stability of important bioactive chemicals. Such studies will be critical for enhancing bioavailability and assessing treatment effectiveness in real-world settings. By combining traditional knowledge with current scientific confirmation, this study increases both plants pharmacological legitimacy and emphasises their importance in the treatment of complicated metabolic disorders.

Conclusion

Plants have a wide array of secondary metabolites which make them potent therapeutic agents. In the present study the antioxidant and antiglycation potentials of two plant materials, *F. asafoetida* and *E. globulus*, were analysed using established methods. Both the plants exhibited significant free radical scavenging power and suppressed the generation of oxidants. However, this oxidative stress suppression was more potently observed with the *E. globulus* oil which is also due to greater amount of phytochemicals as compared to *F. asafoetida*. Similarly, the antiglycation potential was also observed when the plant materials of both the plants were added to glycation system. In contrast to antioxidant property the antiglycation potential was higher in *F. asafoetida*. The presence of more sulfur containing compounds is likely to contribute towards the higher antiglycating potential of *F. asafoetida*. Based on these findings, standardised polyherbal formulations or topical treatments including *F. asafoetida* extract and *E. globulus* leaf oil may be developed to manage oxidative and glycation-related issues in diabetes patients.

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Authors' contributions

PS and AA put forward the conceptualization. PS and DK carried out data curation and formal analysis. AA and FMAL performed funding acquisition, project administration, validation and provided resources. PS, DK and TA carried out investigation and methodology. PS and TA worked out software. AA supervise the entire work. PS, DK and TA visualise the work. PS and AA prepared original draft. PS, DK, AA, TA, FML carried out review writing and editing. All authors have read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare there is not any conflict of interest.

Ethical issues: None

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