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EVALUATION OF ANTIAGING ACTIVITY OF TRIGONELLA CORNICULATA BY USING INVITRO AND INSILICO PHARMACOLOGICAL METHOD

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Abstract:

Kasuri methi is the ethnomedicinal plant (*Trigonella corniculata L*) which is a member of the Fabaceae family. Kasuri methi is also known as sickle fruited fenugreek. In ancient time fenugreek seed and leaves commonly used in Egypt for the treatment of burn. It is currently widely cultivated in central Africa, North America and parts of Australia, with India. Many extracts of each seed or leaf and its active components have been studied for their pharmacological effects and have been reported to have hypocholestrolaemic, antidiabetic, anti-inflammatory, antiulcer, analgesic, antipyretic, CNS-stimulant, antioxidant, wound healing and immune modulatory activity as well as gastro-protective and chemo-preventive activities. This overview consolidates the botanical description, cultivation, phytochemistry, pharmacological activities and medicinal uses of kasuri methi.

Keywords:

Trigonella foenum corniculata “Kasuri Methi”, Pharmacology, Chemical constituents, Medicinal properties, Pharmacological activity.

INTRODUCTION

Aging is a complicated biological process in which functional and structural alterations in a living organism take place over time. Reactive oxygen species is one of the main factors responsible for aging and is associated with several chronic pathologies [1]. Aging is a natural phenomenon that affects entire physiology of an organism. It is an accumulation process of diverse detrimental changes in the cell and tissue with advancing aging. It can also be defined as an increase the probability of death [2]. Aging is the irreversibly progressive decline of physiological function, which eventually leads to age-related diseases, such as cardiovascular diseases, musculoskeletal disorders and arthritis, neurodegenerative diseases, and cancer. These age-related diseases produce a heavy economic and psychological burden for patients, their families, and society as a whole [3]. Skin aging is a part of a natural human “aging mosaic” which becomes evident and follows different trajectories in different organs, tissues and cells with time. While the aging signs of internal organs are masked from the ambient “eyes” the skin provides first obvious marks of the passing time. Skin aging is particularly important because of its social impact. It is visible and also represents an ideal model organ for investigating the aging process. The “biological clock” affects both the skin and the internal organs in a similar way, causing irreversible degeneration.

Kasuri methi is the ethnomedicinal plant (*Trigonella corniculata*) which is a member of the Fabaceae family. Kasuri methi is also known as sickle fruited fenugreek. They were investigated for their potential for use as nutraceuticals [4]. The green leaves and seeds of fenugreek are widely used in food and medicinal applications dating back to ancient times. The leaves and seeds of these both species are used in medicinal and culinary purpose. In ancient time fenugreek seed and leaves commonly used in Egypt for the treatment of burn. It was taken with honey for the treatment of "Dyspepsia" and much other diseases like "Diabetes" and "racket" [5]. It is currently widely cultivated in central Africa, North America and parts of Australia, with India. However, the seeds are sour in taste due to the presence of bitter saponins, which limit their acceptability in foods. It has been possible to decrease the bitter taste of fenugreek seeds by using diverse household processes, such as soaking, germination, boiling, fermentation, etc. Many extracts of each seed or leaf and its active components have been studied for their pharmacological effects and have been reported to have hypocholesterolemia, antidiabetic, anti-inflammatory, antiulcer, analgesic, antipyretic, CNS-stimulant, antioxidant, wound healing and immune modulatory activity as well as gastro-protective and chemo-preventive activities [6-7].

Figure No 1: Plant of *Trigonella corniculata*

This plant is indigenous to the eastern coasts of the Mediterranean and North Africa. According to some experts and scholars, this plant primarily was indigenous to Iran and then was transferred to other areas. Fenugreek is widely grown in India, China, Africa, Algeria, Saudi Arabia, Pakistan, Egypt, Turkey, Ukraine, Spain, and Italy. This plant is frequently exported from India, China, Turkey, and Morocco. Botanically, *Trigonella corniculata*, commonly known as fenugreek, is an annual herbaceous plant in the Fabaceae family. The following detailed descriptions are included:

I. Leaves
- Shape: Trifoliate, ovate-lanceolate.
- Size: 1-3 cm long, 0.5-1.5 cm wide.
- Color: Light green, hairy.
- Arrangement: Alternate, stipulate.
- Leaflets: Three, unequal, terminal leaflet largest

II. Stem
- Shape: Erect, branched.
- Size: 30-60 cm tall, 1-2 cm diameter.
- Color: Green, hairy.
- Texture: Herbaceous, slightly woody at base

III. Flowers
- Type: Papilionaceous.
- Color: White, yellowish-white.
- Size: 1-2 cm long, 1-1.5 cm wide.
- Arrangement: Axillary, solitary, peduncled.
- Calyx: Five-lobed, hairy.
- Corolla: Five-petaled, papilionaceous

IV. Fruit
- Type: Pod.

- Shape: Linear, curved.
- Size: 5-10 cm long, 0.5-1 cm wide.
- Color: Brown, hairy.
- Seeds: 10-20, yellowish-brown, rhomboid

V. Seeds

- Shape: Rhomboid.
- Size: 2-3 mm long, 1.5-2 mm wide.
- Color: Yellowish-brown.
- Surface: Smooth, glossy.

VI. Growth and Cultivation

- Mild Mediterranean climates are suitable.
- Plants mature in about four months.
- Flowering season: midsummer (June to July).
- Requires inoculation with Rhizobium species for optimal growth.
- Low water requirements, making it suitable for dry-land cultivation.

VII. Adaptability

- Distributed worldwide, adapting to various climatic conditions and growing environments.
- Tolerates annual precipitation of 3.8-15.3 dm and annual mean temperature of 7.8-27.5°C [8-12].

MATERIALS AND METHODS:

Collection and authentication of Plant materials:

The source of the plant was self-cultivated in the month of November. The plant material was identified and authenticated from Dr. Lubaina A S professor and Head Department of Botany, Christian College, kattakada. Then, the sample is thoroughly washed with fresh water. Plant is cut into small pieces and were dried in shade for 10 days. The cutting pieces were pulverized to get coarse powder and stored in an airtight container.

Preparation of plant extract:

The extraction process was carried out using maceration method which is a lab equipment for processing certain kinds of solutes. It consists of magnetic stirrer, 100 ml beaker, heating mantle, lid, coarse filter paper. Rinse the beaker using ethanol. Defatted the plant material by using petroleum ether. 100gm powdered plant material was taken in beaker. Then 50ml ethanol and 50 ml water is added to the beaker. The temperature is maintained at 45°C. The lids of the beakers were kept close during the extraction process. The sample were centrifuged for 5mits and filtered using muslin cloth filter paper and extract was remained on the filter paper. The extracts were concentrated to dryness by using hot plate by reduced pressure and controlled temperature. After drying process, extract was weighed and calculated the percentage yield.

$$\text{Percentage Yield} = \frac{w2}{w1} \times 100$$

Where, $w1$ - Weight in grams of dried plant material, $w2$ - Weight in grams of extract obtained.

Preliminary phytochemical screening

Qualitative phytochemical screening was carried out for flavonoids, carbohydrates, tannins, saponins glycosides, amino acids proteins, phenolic compounds, and alkaloids.

IN SILICO MOLECULAR DOCKING STUDY [13]

i. Step 1: Ligand preparation

The chemical structure of the compound was obtained from open chemistry database PubChem. The chemical structures of the compound were downloaded in the 3D conformer format and then converted to sdfmol format. Finally, the input format prepared as pub ligand format and can be visualized in Open babel.

ii. Step 2: Protein preparation

Target molecules such as human growth hormone and extracellular domain 3HHR and human receptor for advanced glycation end products 3O3U by literature survey and respective PDB files such as 3HHR, 3O3U were downloaded from protein data bank. Then the protein is downloaded in crystal structure and after processing convert this to input format.

iii. **Step 3: Protein processing**

Protein structure processing / refinement of protein include removal of hydrogens, water molecules, heteroatoms and bound ligands if any.

iv. **Step 4: Docking analysis**

Docking analysis was done by Auto dock Vina Program in PyRx software. For this installed Auto dock tools (Python 2.7.1, MGL tools l.5.4 and Open babel) and PyRx. Installed Auto dock tools (Python 2.7.1, MGL tools 1.5.4 and Open babel) and PyRx.

v. **Step 5: Molecular visualization**

PyMOL is software used for the protein preparation and molecular visualization. It produces high quality 3D images of protein.

IN VITRO STUDY OF ANTIAGING ACTIVITY

Reactive Species of oxygen (ROS) detection:

Procedure

Cell seeding:

- Seeded 2 × 105 L929 cells in petri dishes and maintained the cells in Dulbecco's modified Eagle medium (DMEM) with 10% FBS (Fetal Bovine Serum) at37 °C.
- After the cells attained the required confluency, the cells were divided into four categories. (1) Untreated control and (2) Cells exposed to IC 50 concentration (50 µM) of H_2O_2 (3) Cells exposed to 50 µg/mL sample and 50 µM H_2O_2 and (4) Cells exposed to 100 µg/mL sample and 50 µM H_2O_2. Cells were then incubated at37 °C for 24 hours.
- After the incubation period, cells of different groups were subjected to ROS staining using the dye DCFH-DA and were observed under the blue filter of an inverted phase contrast fluorescent microscope (Labomed, TCM 400, USA).

Preparation of the DCFH-DA solution

- Dissolve 4.85 mg of DCFH-DA in 1 mL of dimethyl sulfoxide (DMSO) to make a 10 mM stock solution.
- Dilute the stock solution with pre-warmed DMEM into 10 µg working solution right before adding it to the wells.
- Vortex the working solution for 10 s.

DCFH-DA staining

1. Remove the drug containing medium and wash once with DMEM.
2. Add 500 µL of the DCFH-DA working solution into each well and incubate at 37 °C for 15 min in dark.
3. Remove the DCFH-DA working solution. Wash once with DMEM and 2 times with1× phosphate-buffered saline (PBS).
4. Observe under an inverted phase contrast fluorescence microscope [14].

(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

Procedure

- The cells (10,000 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37°C and 5% CO_2 environment in the incubator for 24 hours.
- The test samples were prepared in DMSO (10 mg/mL) and filter sterilized using 0.2 µm Millipore syringe filter.
- The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100 µg/mL respectively.
- Untreated wells were kept as control.
- All the experiments were done in triplicate and average values were taken in order to minimize errors.
- After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells.
- The plates were further incubated for 2 hours for the development of formazan crystals.
- The supernatant was removed and 100 µl DMSO (100%) were added per well.
- The absorbance at 570 nm was measured with micro plate reader.
- Three wells per plate without cells served as blank.
- All the experiments were done in triplicates.

- The cell viability was expressed using the following formula [15-16]

$$\text{Percentage of cell viability} = \times 100$$

RESULTS AND DISCUSSION

Percentage yield

NAME OF THE PLANT	PLANT PARTS USED	METHODS OF EXTRACTION	SOLVENT USED FOR EXTRACTION	PERCENTAGE YIELD(%W/W)
Trigonella corniculata	sprout	Maceration	Ethanol	10.5%W/W

Table No 1: Percentage yield of plant extract of *Trigonella corniculata*

Preliminary phytochemical screening

SL.NO.	NAME OF THE CONSTITUENTS	NAME OF THE TEST	EETG
1	Flavonoids	Shinoda test	+
		Lead acetate test	+
		Alkaline test	+
2	carbohydrates	Fehling's	+
3	Proteins and amino acids	Xanthoproteic test	+
4	Alkaloids	Dragendorff's test	+
5	Tannins	Ferric chloride test	+
6	Phenolic compounds	Lead acetate test	+
7	Saponin glycosides	Foam test	+

Table No 2: preliminary phytochemical screening of ethanolic plant extract of *Trigonella corniculata.*

In silico study of *Trigonella corniculata*:

In silico docking studies facilitate interaction among the components in a system and mathematical and computed models are established and predict the interaction between ligand and target molecules. From various literature reviews founded that Trigonella corniculata possess bi-flavones i.e. qucertin and rutin which has the ability to produce anti-aging. Qucertin and rutin shows binding affinity with 3HHR, 3O3U receptors.

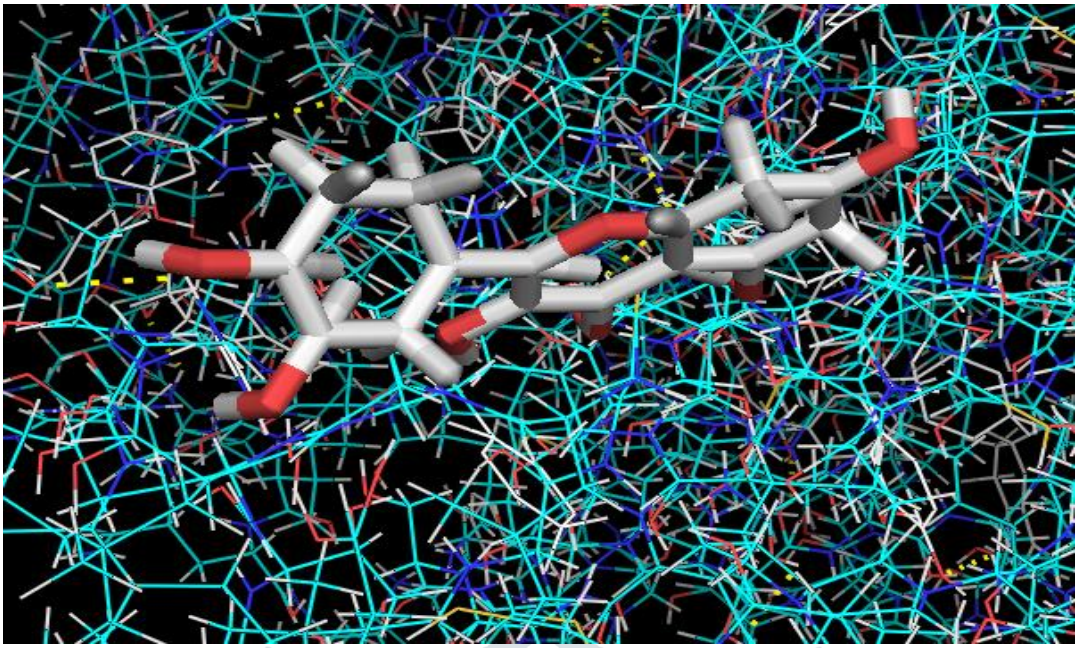

Figure No 2: Docking images of Qucertin with receptor 3HHR

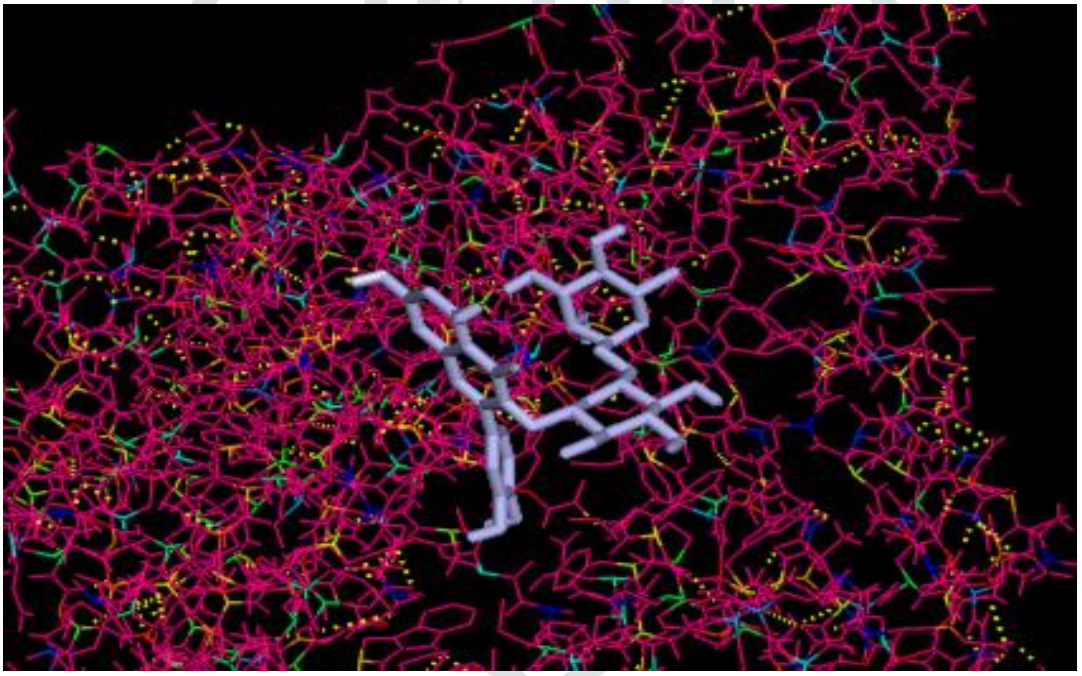

Figure No 3: Docking images of Rutin with receptor 3O3U

RECEPTORS	COMPOUND	NUMBER OF HYDROGEN BOND INTERACTIONS	BINDING AFFINITY(KCAL/ MOL)
3HHR	Qucertin	5	-7.7
3O3U	Rutin	4	-9.9

Table No 3: Hydrogen bond interaction and binding affinity of qucertin and rutin with receptors

In vitro* study of *Trigonella corniculata

MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay:

At increasing concentrations, ethanolic extract of plant of *Trigonella corniculata* decreasing cytotoxicity was observed in L929 cytotoxic fibroblast cells administered with different concentrations of the sample. Thus, the sample studied non-cytotoxic to normal cell line. The sample was found to be non-cytotoxic to normal cell lines.

CONCENTRATION (MG/ML)	PERCENTAGE OF CYTOTOXICITY (%)
6.25	99.16
12.5	98.56
25	98.15
50	96.90
100	94.83
IC 50	>100 µg/mL

Table No 4: Effect of ethanolic extract of *Trigonella corniculata* in MTT Assay

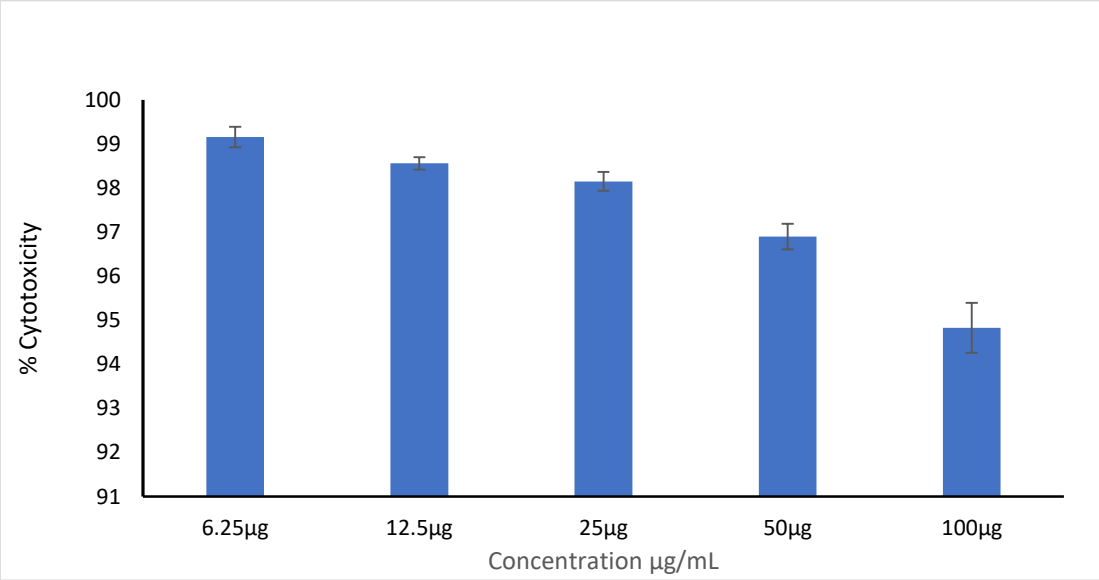

Figure No 4: Graphical representation of MTT Assay.

Reactive Oxygen Species (ROS) Detection:

Control cells: Very low fluorescence, indicating basal levels of reactive oxygen species production in L929 normal fibroblast cell line.

H_2O_2 (IC 50 concentration – 50 µM): Higher levels of fluorescence were observed at this concentration. This indicates significant elevation in levels of ROS production in L929 normal cells due to sample addition.

Sample (50 µg/mL) + H_2O_2: Relative reduction in fluorescence intensity compared to H_2O_2 treated cells was observed.

Sample (100 µg/mL) + H_2O_2: Relative reduction in fluorescence intensity compared to H_2O_2 and sample (50 µg/mL) treated cells was observed.

Cell images shows that treatment with hydrogen peroxide has increased the levels of ROS in cells which resulted in change in cell morphology from elongated in untreated cells to rounded in H_2O_2 treated cells. With KM sample pretreatment ROS levels were found to be decreased with respect to H_2O_2 treated cells. This indicates the anti-oxidant potential of the sample and also its potential application in preventing cellular damage caused due to oxidative stress and free radicals.

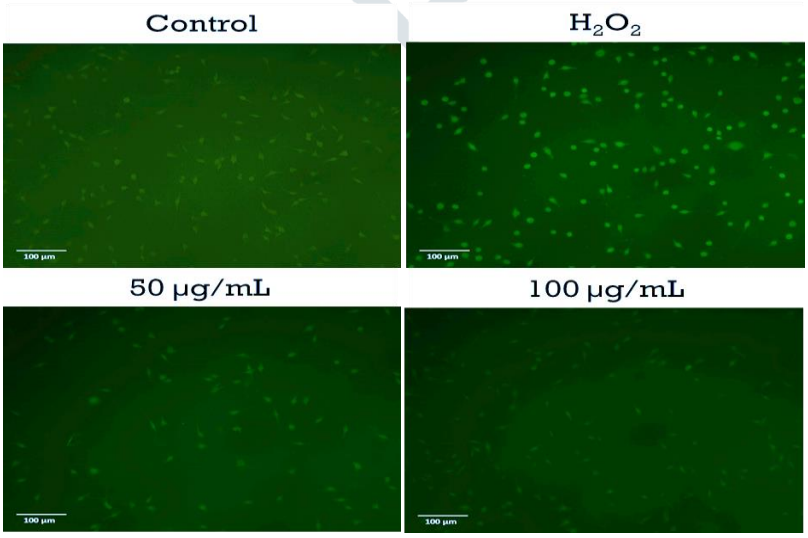

Figure No 6: Fluorescent Images of ROS detection

DISCUSSION

Kasuri methi (*Trigonella corniculata*), a member of the Fabaceae family, has long been used in food and medicine. Its leaves and seeds have been traditionally used for treating burns, dyspepsia, diabetes, and rickets. Though bitter due to saponins, its taste can be improved through processes like soaking and fermentation. The plant is cultivated worldwide, including in India. A study collected *Trigonella corniculata* from Tamil Nadu, processed it into a powdered form, and extracted its components using an ethanolic maceration method. Phytochemical analysis confirmed the presence of flavonoids, phenolic compounds, tannins, proteins, amino acids, saponins, and alkaloids, which have medicinal properties like anti-aging, antioxidant, anti-inflammatory, and anticancer effects. Molecular docking studies revealed that flavonoids Quercetin and Rutin in *Trigonella corniculata* show strong binding affinity with aging-related receptors, suggesting potential anti-aging benefits. The MTT assay confirmed the extract's non-cytotoxicity. ROS detection showed its potential in regulating oxidative stress, which is crucial for cellular function.

The study highlights the plant's phytoconstituents and supports its traditional use in anti-aging and antioxidant treatments.

CONCLUSION

The present study was done to evaluate the *in vitro* and *in silico* anti-aging activity ethanolic plant extract of *Trigonella corniculata*, seen in tropical regions which possess medicinal benefits. The plant *Trigonella corniculata* is one of the traditional medicinal plants in the world wide which is used as an edible plant and also have the therapeutic active compound. The more research work has been already done in this plant. From the literature survey in various region, population used this plant as an anti-oxidant, hepatoprotective action, anti-cancer activity and anti-diabetic activity. This activity is mainly due to the presence of phytochemicals. The ethanolic plant extract contain the various phytochemicals such as flavonoids, phenolic compounds, tannins, proteins and amino acids, saponins and alkaloids.

Due to the presence of active constituent especially flavonoids, pharmacological activity is identified through the *in vitro* assay and *in silico* methods. Finally, this study indicates the active constituents is responsible for the anti-aging activity.

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