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Research Article

Screening of elastase and tyrosinase inhibitory activity of *Trachyspermum ammi, Anethum graveolens & Foeniculum vulgare*

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Abstract

The science of Ayurveda has utilized many herbs and floras to make cosmetics. The natural content in the botanicals does not cause any side effects on the human body. Indian medicinal plants were screened to estimate the enzymatic activity. To identify Anti-Aging and skin- whitening, Plant extracts were investigated for their tyrosinase inhibitory activity and elastase with potential use of raw material for cosmetics use. In tyrosinase inhibition assay, three plant extracts of *Trachyspermum ammi*, *Anethum graveolens & Foeniculum vulgare* showed 42.25%, 25.37% & 20.32% activity respectively as compared to Kojic Acid standard, which showed 89.437% activity are shown to have potent tyrosinase inhibitors. Elastase inhibition assay was utilized for this evaluation and the seeds of plants *Trachyspermum ammi*, *Anethum graveolens & Foeniculum vulgare* showed the activity of about 61.96, 56.84% & 48.72% respectively. The activity was less than that of the Standard Areca catechu. Results of these medicinal plants suggest that plants possessing several biological activities may be potent inhibitors of the process involved in pigmentation increases and aging. Further probe will focus on in vivo assays and the chemical identification of the major active components responsible for anti-aging and whitening.

INTRODUCTION

Skin is the largest and imperative organ of the body, protecting it from damage caused by direct contact with the outside of the environmental factors that injure the skin, ultraviolet (UV) irradiation is the most common and pernicious. It leads to alterations in the composition of the skin, including the accumulation of elastic fibres (Dawe *et al*., 2003), collagen reduction and degeneration (Korac & Khambholja, 2011) and deposition of glycosaminoglycans (Prity *et al.*, 2021)¨ The key enzyme Tyrosinase that catalyses melanin synthesis in plants, microorganisms and mammalian cells. Melanin biosynthesis inhibitory compounds are very useful for skin whitening agents in cosmetics trials on tyrosinase inhibitors in the Cosmetics and Pharmaceuticals industry have been able to avoid over production of melanin in epidermal layers (Masum, *et al.*, 2019; Pillaiyae *et al.*, 2017). The ongoing attacks of free radicals damage the elastin and collagen fibers. The skin protects itself against these impairments with the aid of radical scavengers particularly in collagen and elastin fibrils. In addition, elastase plays a decisive role in the control of inflammatory processes. Under physiological conditions, the elastase activity is controlled by inhibitors. The objective of the work was to unfold strategies and in-vitro screening methods, extraction procedures, bioassay-directed isolation and characterization of natural products. The current study work was undertaken for screening and to evaluate the tyrosinase inhibitory i.e. Skin Whitening & Elastase inhibitory i.e. Anti-Ageing potential of *Trachyspermum ammi*, *Anethum graveolens*, *Foeniculum vulgare*. Physicochemical assessment of foeniculum vulgare is evidence for

its different in vitro pharmacological properties like antimutagenic, anti-inflammatory, antiviral, antimicrobial, antispasmodic etc. It can treat more than 40 types of disorders (Badgujar *et al*., 2014). Antioxidant potential of anethum graveolens along with its phytochemical contents and enzyme inhibition properties are studied in samples grown under organic and conventional agriculture environments (Erdogan *et al*., 2013). Ajwani is the source of different volatile oils like thymol, p-cymene, c-terpinene, and α- and β-pinene (Hafiz & Hafiz., 2020)

MATERIALS & METHODS:

Plant Materials & Solvent Extract

Preparation of Plant Seed Extracts of *Trachyspermum ammi, Anethum graveolens, Foeniculum vulgare* were purchased from the local market and it was identified & authenticated by scientist "Priyanka A. Ingle" from the Botanical survey of India (BSI). The sample materials were brought to the laboratory and were shade dried at room temperature for a few days. The dried seed samples were analysed for percentage moisture content.

Extraction Method

In the present study sample powder of formulation 20g was weighed and Soxhlet extracted using 250ml methanol (industrial grade solvent). The temperature was set at 65ºC. The filtrate was collected after 7 cycles which approximately carried out in 24 hours and was purified using rotavapor. The extract was collected in a petri plate and left overnight for complete evaporation of the solvent. The extract of formulation was thus obtained and purified. This extract was stored in a cool dark place and used for all the further experiments.

1.1 Tyrosinase Inhibition Assay

Each Plant was evaluated for Tyrosinase inhibition by measuring its effect on tyrosinase activity using a 96-well reader (Power Wave XS; BioTek). The reaction was carried out in a 50mM potassium phosphate buffer (pH 6.8) containing 20mM L-tyrosine and 312 U/ml mushroom tyrosinase at 30ºC. The reaction mixture was pre-incubated for 15 mins with tyrosinase. The reaction mixture with the corresponding solvents (without plant material) served as control and without enzymes served as blank. The change in absorbance at 492 nm was measured.

1.2 Elastase Inhibition Assay

The activity of porcine pancreatic elastase [PPE (Type IV); Sigma Chem. Co.) was studied using N-Suc-(Ala)3-nitroanilide (SANA) as the substrate, and the release of nitro aniline at 410 nm was measured. The reaction was carried out in 140µl of 200 mM Tris-HCl buffer (pH 8.0) containing 10µl of 5 mM N-Suc-(Ala)3- nitroanilide and 10µl of 10 µg/mL elastase. 20µl of Plant extract was added to the reaction mixture to reach a final concentration of 1mg/mL, and elastase inhibition was assessed at 25°C. The reaction mixture was preincubated for 20 min before adding the substrate and 30 mins after adding substrate. The change in absorbance was measured at 410 nm using a 96-well reader.

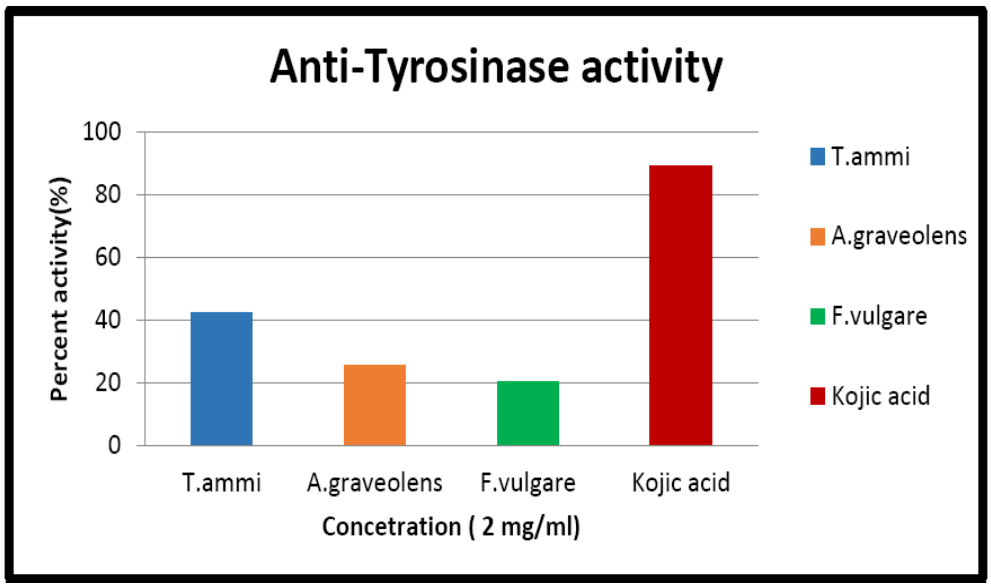
RESULTS:

The global market has seen relentless demand for natural substances, such as plant extracts, that can be used for depigmenting, anti wrinkle, and other cosmeceutical purposes(Pillaiyae et al., 2017),(Badgujar et al., 2014). Moreover, plant extracts with an inhibitory effect on melanin formation and elastase activity may be good choices for cosmetic purposes because of their relatively low incidence of side effects. At present, cosmetic preparations have been developed using plant seed extracts such as *Trachyspermum ammi, Anethum graveolens, Foeniculum vulgare* seeds). We evaluated the effects of these medicines on elastase and tyrosinase activity. As shown in the (Table.1) The plant extracts of *Trachyspermum ammi, Anethum graveolens & Foeniculum vulgare* showed 42.25%, 25.37% & 20.32% activity respectively as compared to Kojic Acid standard, which showed 89.437% activity in (Fig 1) are shown to have potent tyrosinase inhibitors. Whereas in (Table 2) shows the activity of about 61.96, 56.84% & 48.72% respectively. The activity was less than that of the Standard Areca catechu as shown in (Fig 2) Therefore, we evaluated their effects on tyrosinase & elastase enzyme as its percentage inhibition activities.

Table 1: Percent Anti- Tyrosinase activity of *Trachyspermum ammi (Carom)*, *Anethum graveolens (Dill)*, *Foeniculum vulgare* (Fennel) Ethanolic Extract

Name of the Plant Seed	Tyrosinase Concentration (mg/mL)	Test	Blank	X	% Activity
Carom	**2**	0.913	0.41	0.503	42.2502
Dill	**2**	1.049	0.399	0.65	25.3731
Fennel	**2**	1.003	0.309	0.694	20.3214

Fig 1: Anti- Tyrosinase activity of *Trachyspermum ammi (Carom)*, *Anethum graveolens (Dill)*, *Foeniculum vulgare*'s (Fennel) Ethanolic Extract



Tyrosinase Inhibition assessment:

Percent Inhibition (%) = [(A-B)/A] × 100 where,

A is absorbance at 475 nm without plant extract,

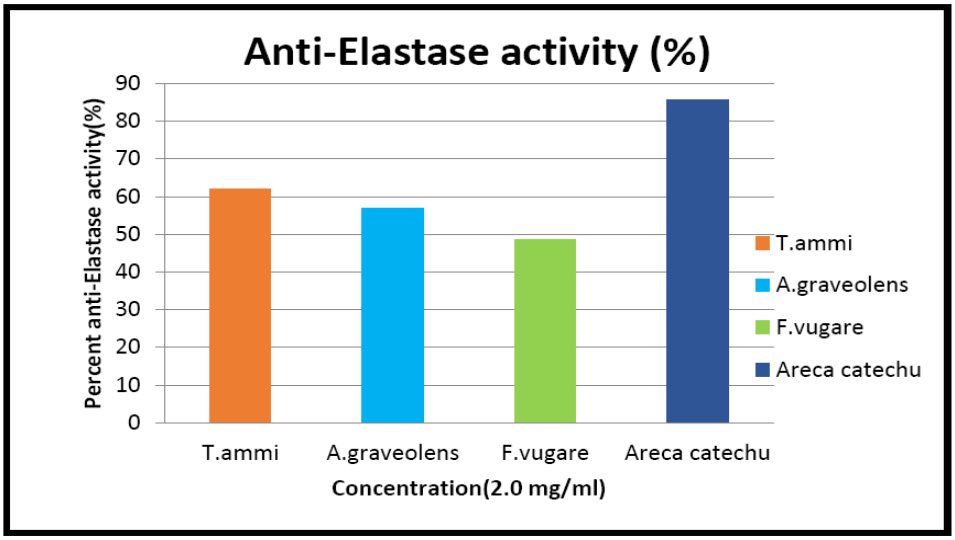
B is the change in absorbance at 475 nm with plant extract……..(Eq. 1)

Table 2: Percent Anti-Elastase activity of *Trachyspermum ammi (Carom)*, *Anethum graveolens (Dill)*, *Foeniculum vulgare*'s (Fennel) Ethanolic Extract

Name of the Plant Seed	Concentration (mg/mL)	Test	Blank	X	% Activity
Carom	2	0.315	0.226	0.089	61.9658
Dill	2	0.313	0.212	0.101	56.8376
Fennel	2	0.273	0.153	0.12	48.7179

O.D: Optical Density

Fig 2: Graph of Anti-Elastase activity of *Trachyspermum ammi (Carom), Anethum graveolens (Dill), Foeniculum vulgare*'s (Fennel) Ethanolic Extract



Elastase inhibition assesment :
Inhibition (%)= [(A-B)/A] × 100,
where A is absorbance at 410 nm without plant extract,
B is the change in absorbance at 410 nm with plant extract……..(Eq. 2)

CONCLUSION:

The present study was carried out for the preliminary screening and evolution of phytochemicals, Antioxidant activity, Anti-inflammatory activity, anti-tyrosinase activity (skin whitening), Anti-elastase activity (anti-ageing) of different plant extracts (*Trachyspermum ammi, Anethum graveolens, Foeniculum vulgare* seeds).

In comparison with the synthetic compounds that are currently available to us and taking into consideration their side-effects, it is completely essential to develop or to discover newer drugs and remedies with low or no side effects and increased potency. This aim could be achieved through plants *Trachyspermum ammi, Anethum graveolens, Foeniculum vulgare* extract could be an ideal candidate for this purpose.

In this part of the study, only their fundamental properties were studied in addition to enzymatic assays in-Vitro. The next part of the study includes the extraction and purification of the present phytochemicals by various advanced technologies like HPLC, LC-MS, etc., Once the products are being retrieved, in-Vitro studies on cell lines will be done with assays like Cell Cytotoxicity Assay, Wound-healing, etc., Further the purified plant extracts could be exploited for efficacy testing and cosmetic evaluation before pre-clinical studies. All this clearly opens up a huge challenge for conservationists, researchers, industry and farmers to manage one of the most important natural resources wisely.

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