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Ethanollic extract of *ocimum kilimandscharicum linnleaves*: phytochemical and *in vitro* pharmacological activity

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Abstract

Using an *in vitro* model, the anti-thrombolytic efficacy of ethanolic extracts of *Ocimum kilimandscharicum Linn* was investigated. The researchers discovered that different concentrations of the extract had significant anti-thrombolytic activity in a dose-dependent manner, which was comparable to a standard drug. As a result of the presence of flavonoids and polyphenols in the plant extract, it can be concluded that it has a promising future in the treatment of thrombosis. This knowledge will be useful in the clinical development of thrombolytic therapeutics by identifying more potent anti-thrombolytic principles from natural resources.

Keywords: *Ocimum kilimandscharicum Linn*, Ethanolic extract, anti-thrombolytic activity.

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Introduction

Nature has always served as a shining example of the remarkable phenomenon of symbiosis. The treatment of human disease has traditionally relied on natural products derived from plants, animals, and minerals. According to current estimates, nearly 80% of people in underdeveloped countries still rely on traditional medicine, which is mostly based on plant and animal species [8], for their primary health treatment.

Herbal remedies are in high demand right now, and their popularity is growing by the day. Around 500 medicinal plants have been referenced in ancient literature, and around 800 plants have been used in indigenous medical systems. India is home to a large number of medicinal plants that are employed in traditional medicine. According to the World Health Organization, about 80% of the world's population in developing countries is completely reliant on medicinal

Plants for their primary healthcare. Plants are directly or indirectly responsible for almost 25% of prescribed medicines in industrialised countries [2]. Plants are a key source of novel medicinal compounds. Almost every new drug on the market today is either a natural product or a semi-synthetic derivative derived from one. The presence of a combination of unique chemical features and potent bioactivities in the secondary metabolites of these plant sources has long contributed to the development of therapeutic molecules [3].

Because of its cultural acceptability, compatibility with the human body, and lack of side effects, herbal medicine is still the primary health care choice for around 75–80 percent of the world's population, primarily in developing nations. Plant extracts or active chemicals derived from or modelled on plant compounds are believed to be found in around a quarter of all prescribed medications. Physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine are only a few instances of what medicinal plants have given us past 4. Medicinal plants remain an essential medicinal assistance in the treatment of human illnesses. Over the previous 2500 years, highly strong traditional medical systems such as Chinese, Ayurvedic, and Unani have been born and practised, primarily on the eastern continent. These plants contain chemicals that can be

exploited for therapeutic purposes, including drug synthesis precursors [5].

Kingdom's great diversity represents an enormous reservoir of compounds with potential therapeutic value [6].

Activity of the Thrombolytic System

Thrombosis is a deadly condition marked by the formation of a blood clot (thrombus) in the circulatory system as a result of an imbalance in the body's homeostatic system. It produces vascular blockage, which can lead to catastrophic consequences such myocardial or cerebral infarction, as well as death [7]. Thrombosis is the clotting of blood vessels. A blood clot in a blood vessel's presence venous thrombosis and Arterial thrombosis are the two most common types of thrombosis, based on where the clot forms.

Anticoagulant and antiplatelet aggregation medicines are used to prevent and treat thrombosis, while thrombolytic treatments are mostly used to treat thrombus breakdown.

Antiplatelet drugs like clopidogrel and aspirin are frequently used in clinical practise, although they have a variety of side effects. As a result, research has been performed to find naturally produced antiplatelet aggregation that has fewer adverse effects. Plants have been employed by man for their amazing healing and pain-relieving qualities since the dawn of time. Currently, the medical community is increasingly turning to plants to treat a variety of illnesses and pains, primarily to avoid the severe side effects associated with the use of contemporary drugs [1].

Antiplatelet action derived from natural sources could be important. When compared to synthetic drugs, antiplatelet activity from natural sources may play a key role in the treatment of diseases with minimal side effects. As a first start in this manner, we focused our efforts on finding herbal remedies and selecting medicinal plants from the Lamiaceae family, specifically *Ocimum kilimandscharicum* Linn, for their in vitro antiplatelet activity.

Collection of plant materials

In March 2017, plant material of *Ocimum kilimandscharicum* Linn was taken from the local market zone of Tirupathi, authenticated by recognised botanist Dr.K.Madhavachetty, and placed in the same institute as specimen voucher no 2127.

Plant extract preparation

Ocimum kilimandscharicum Linn leaves were shade dried and coarsely pulverised. Using a weighted amount of 70gms of powder, the extraction procedure was carried out using the continuous hot percolation method with the use of a soxhlet apparatus, while adhering to all of the practical parameters listed below. Solvent selection is critical for thorough extraction of needed elements and

obtaining a high percentage yield; with this goal in mind, ethanol was chosen as the extraction solvent.

Streptokinase is a common antibiotic (SK)

5 ml 0.9 percent sodium chloride (NaCl) was added to the commercially available lyophilized S-Kinase™ (Streptokinase) vial (Batch no: VEH 09, Popular Pharmaceutical Ltd., Bangladesh) of 15, 00,000 I.U. This solution was utilised as a stock for the in vitro thrombolysis assay, with 100 l (30,000 I.U) being employed. The method of [9] was used to test the in vitro clot lysis activity of *Ocimum kilimandscharicum*. 12 mL venous blood was taken from healthy volunteers (n = 5) and divided into two sterile micro-centrifuge tubes (2 mL each). The micro-centrifuged tubes were incubated at 37°C for 45 minutes after being micro-centrifuged. After the development of clot, serum was entirely withdrawn from the tubes (taken out without disrupting the clot formed) and each tube bearing clot was again weighed to estimate the weight of the clot (clot weight = weight of clot containing tube – weight of tube alone).

Each micro-centrifuge tube containing clot was carefully labelled, and 100µ l of the plant extract was added to the tubes at various concentrations (2, 4, 6, and 8 mg/ml, respectively). 100 µ l of streptokinase was used as a positive control, and 100 µ l of sterilised distilled water was used as a negative non-thrombolytic control. Water was introduced to each of the numbered control tubes independently. The tubes were then incubated at 37°C for another 90 minutes to check for clot lysis. Following incubation, the collected fluid was withdrawn from the tubes and weighed again to determine the weight difference following clot disruption. Finally, the weight difference was calculated, and the result was reported as a percentage of clot lysis using the equation below.

$(\text{wt. of released clot} / \text{clot wt.}) / 100 = \text{percent of clot lysis}$

B) Activity against platelet aggregation

Blood from normal heparin-free blood bank donors was centrifuged (1000 rpm for 5 minutes) to produce platelet rich plasma (PRP). For every 8.5 ml of blood, 1.5 ml of acid citrate dextrose was employed as an anticoagulant. PRP was collected and placed in siliconized glass cuvettes. The reference was platelet poor plasma (PPP) obtained by centrifugation (3000 rpm for 5 min). The cuvettes were incubated for 5 minutes at 37°C. Different concentrations of medication EEOK 0.5 ml (100, 200, 400g/ml) solution and platelet rich plasma 0.5 ml are present in the reaction mixture. These reaction mixtures were kept at 37°C for 2 minutes with constant stirring. The absorbance was measured at 414 nm after adding 0.5 ml of ADP solution and incubating for 4 minutes. The following formula was used to calculate ADP-induced platelet aggregation. The reference standard was commercial heparin (20 g/ml). The maximum amount of aggregation was measured.

$$\% \text{ ADP induced platelet aggregation} = \frac{(T1-T2) - (T1-T3)}{(T1-T2)} \times 100$$

Where, T1= ADP + Platelet, T2= ADP + Heparin +Platelet and T3= ADP + *EEOK* +Platelet

Analytical Statistics

The data were analysed with the Graph pad instant demo version and a p value of 0.05 was judged statistically significant. For each parameter in each group, the mean and standard deviation were computed.

Experimental Investigations

Table 01: *in vitro* Thrombolytic activity of the *EEOK* and comparison with standard drug Streptokinase;

S.no	Treatment	% clot lysis
1	Extract(2 mg/ml)	3.16±0.73
2	Extract(4 mg/ml)	6.06±1.058
3	Extract(6mg /ml)	9.60±0.626
4	Extract(8mg /ml)	12.740±0.847
5	Extract(10mg /ml)	16.19±0.691
6	Streptokinase	48.24±3.739
7	Control(water)	7.07±0.45

All values were expressed in mean ± S.E.M

Table 02: *in vitro* anti -platelet activity of the *EEOK* and comparison with standard drug Heparin;

S.no	Treatment	% inhibition of platelet aggregation
1	Extract(100 mg/ml)	24.63±2.56
2	Extract(200mg/ml)	39.19±3.44
3	Extract(400mg /ml)	50.60±4.54
4	Heparin	78.99±2.94
5	Control	-----

All values were expressed in mean ± S.E.M

Discussion of Results

Thrombolytic Activity

Thrombolytic activity: The primary goal of this study was to compare the anti-thrombolytic activity of plant extracts to that of a control group. The measured thrombolytic % indicates that the ethanolic extract of *Ocimum kilimandscharicum* leaves has a good thrombolytic action. When compared to streptokinase, it has a higher percentage of thrombolysis and is different from the control. The extract of *O.kilimandscharicum* leaves has a thrombolysis percentage of 16.190.691 percent, while streptokinase has a thrombolysis percentage of 48.243.739 percent. As a result, it can be classified as a potent anticoagulant.

Anti-platelet activity

The results reveal that *EEOK* extracts have efficient antiplatelet activity in a dose-dependent manner, with maximum activity at a concentration of 250g/ml. However, as compared to conventional heparin at a concentration of 20g/ml, the prevention of platelet aggregation was lower. The flavonoids in the ethanolic extract may have prevented platelet adhesion and aggregation, as well as cytoplasmic calcium release, which

enhances the release of ADP. Flavonoids appear to reduce platelet aggregation by causing an increase in cyclic AMP levels in platelets by either stimulation of adenylate cyclase or inhibition of cAMP phosphodiesterase activity⁵. In order to elucidate the inhibitory mechanism of *EEOK*, the tests in this work were developed to evaluate the antiplatelet activity of various fractions of *EEOK*. Was an attempt to figure out how flavonoids inhibit platelet aggregation. Further research suggests that those effects are attributable to phospholipase C inhibition, which results in reduced phosphoinositide breakdown, followed by inhibition of thromboxane A₂ production, and finally reduction of [Ca²⁺] mobilisation of platelet aggregation triggered by agonists. The extract of *O.kilimandscharicum* leaves has a platelet aggregation percentage of 50.604.54 percent, while Heparin has a platelet aggregation percentage of 78.992.94 percent. As a result, strong anti-platelet activity may be deduced.

Conclusion

The antiplatelet activities of *Ocimum kilimandscharicum* Linn's ethanolic extract were investigated to learn more about the plant's biological activities. As a result, we discovered that the *EEOK* reduced platelet aggregation. Despite the fact that the effects of *EEOK* have been shown *in vitro*, these findings suggest that *EEOK* could be used as a crude medicine. The plant exhibited strong enzyme inhibitory activity, necessitating the extraction, purification, and characterisation of the compounds responsible for the inhibitory activity in order to use an anti-platelet drug.

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