

Synergistic anticoagulant effect of *Ocimum sanctum* and *Zingiber officinale* in Blood Samples of Normal Individuals

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ABSTRACT

Searches for substances with anticoagulant activity are frequent, and medicinal plants have been considered interesting by some researchers since they are frequently used in popular medicine as remedies for dissolving the blood clots. The aim of this study was to verify the synergism between two plant extracts used as anticoagulant drugs— *tulsi (Ocimum sanctum)* and *ginger (Zingiber officinale)* against blood coagulation, and for this purpose, the synergistic effect of *O. sanctum* (Tulsi) and *Z. officinale* (ginger) aqueous extract on clotting time. Coagulation activity of *O. sanctum* and *Z. officinale* was measured by capillary glass method on blood samples collected from regular blood donors. The blood was tested against different ratio of aqueous extract of *O. sanctum* and *Z. officinale* as follows: 0:0, 0:1, 1:0, 1:1, 1:2, 2:1. Result shows the aqueous extract of *O. sanctum* and *Z. officinale* prolonged the clotting time. The extracts; tulsi, and ginger presented the highest synergism rate with anticoagulant drugs.

INTRODUCTION

In a constant attempt to improve their quality of life, men have used plants as source of food, shelter, clothing, medicine, cosmetics, and for seeking relief from hardship of life. Some plants are known as medicinal because they contain active substances that cause certain reactions, from relenting to the cure of diseases, on the human organism (Silva Junior et al. 1994). Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups (Di Stasi 1996); Anticoagulants are chemical substances that prevent or reduce blood coagulation as well as prolonging the clotting time and are also known as blood thinners. Anticoagulants are used in therapy for thrombotic disorders¹.

Based on World Health Organization (WHO), about 80% of total world's populations especially in Asia are dependent on traditional medicine for primary healthcare. Herbal plants are considered as important sources of medicine from which large number of therapeutic

drugs are obtained due to their chemical diversity². In India, the herbal drug market is about one billion and the export of plant based crude drugs about 80 million³. The phytochemicals from herbal plants are found to have biological activities such as anticoagulant properties. Therefore, the use of herbal medicine provides an alternative to overcome the limitations of available anticoagulants such as warfarin and heparin which have bleeding complication, as well as uncertainty of the newer anticoagulant drugs dosing in some patient populations such as patient with underlying chronic diseases⁴.

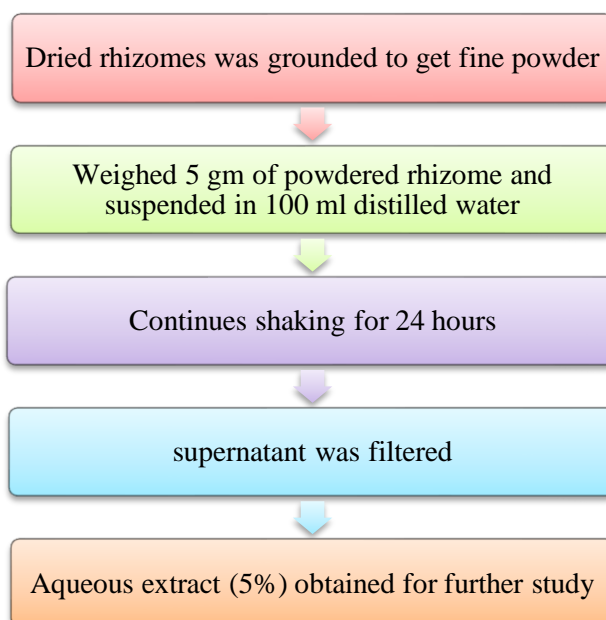
Among the various plants, holy basil and ginger are used for the study of synergistic anticoagulant property on blood samples. The plants are selected for the study as they are cheap, less side effects and easily available. According to “Rehan HMS” and “TajEldin IM” the plants *O. Sanctum* and *Z. Officinale* respectively, demonstrated that aqueous extract of both the plants possesses anticoagulant properties through prevention of coagulation process and clot formation^{5,6}. The phyto-pharmacological activity of the plants is really inspiring and knowledgeable.

MATERIAL USED

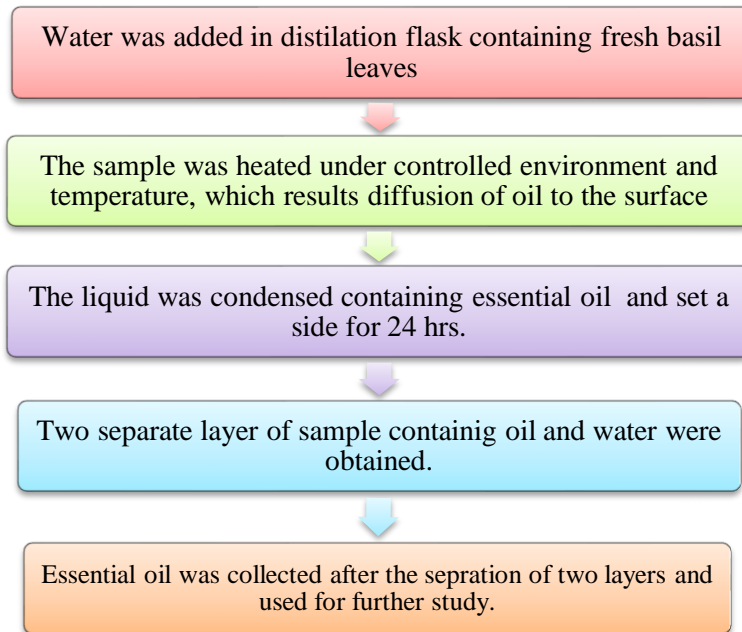
The leaves of the plant *Ocimum sanctum* were collected from the local garden of Pharmacy College campus Itaura, Chandeshwar, Azamgarh. The leaves were examined by Prof. Nawal Kishore Dubey (FNASc, FNAAS, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005). The rhizomes of *Zingiberofficinale* is purchased from local market of Itaura, Azamgarh (near Pharmacy College Azamgarh). The rhizomes were examined by Prof. Nawal Kishore Dubey (FNASc, FNAAS, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005). The veinous blood samples from four different blood groups (O +ve, A +ve, B +ve and AB +ve) are collected from the right arm of healthy blood donors using sterile syringes.

METHODOLOGY

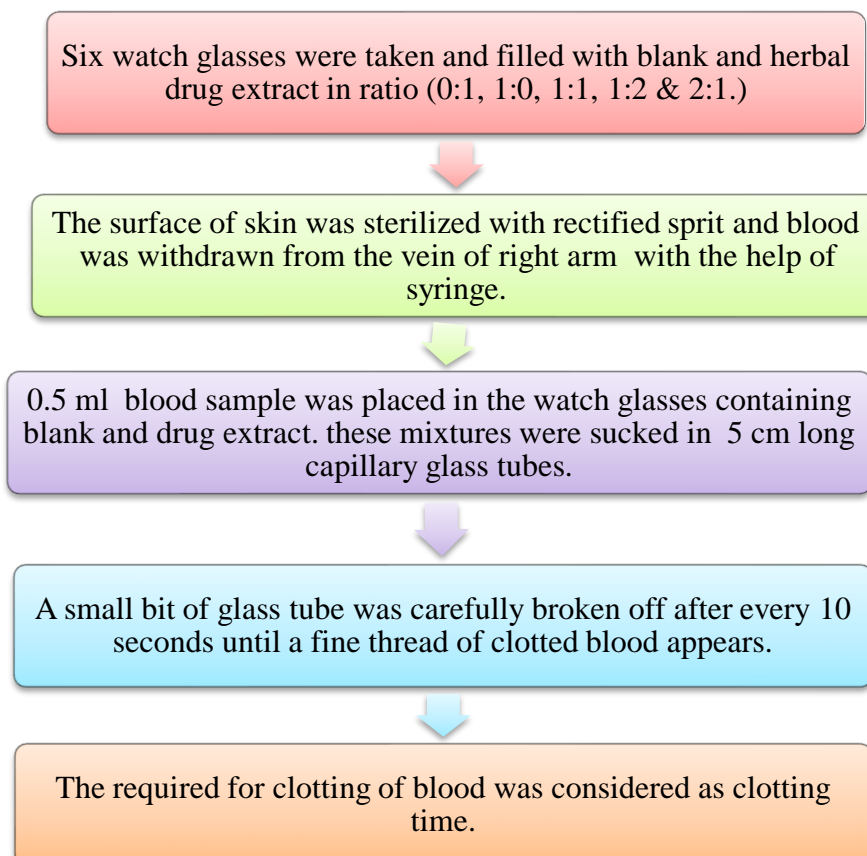
Ginger extraction



Basil extraction



Determination of clotting time

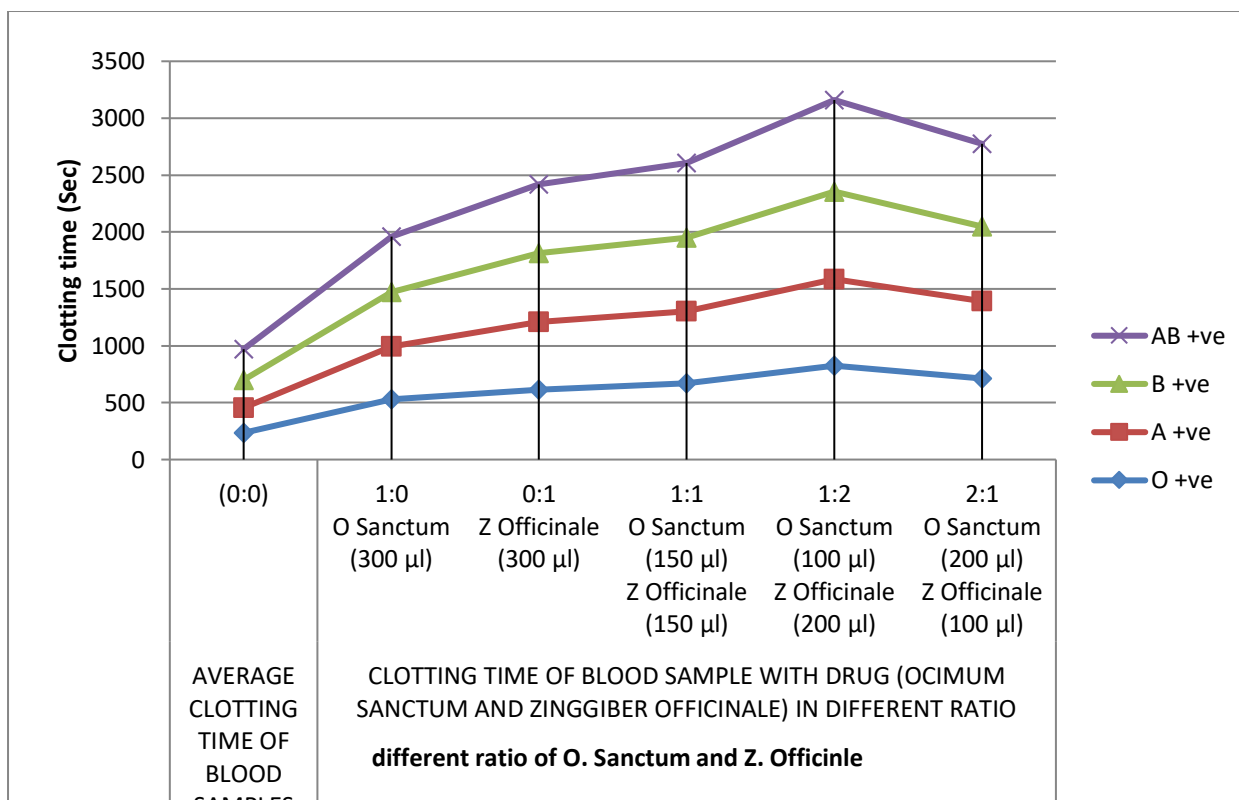


RESULT

In this study the Synergistic Anticoagulant Effect on aqueous extract of *Ocimum sanctum* and *Zingiber officinale* on blood samples are tested by clotting time determination using Capillary Glass Method on the blood samples of healthy individuals having different blood groups (O +ve, A +ve, B +ve and AB +ve). The clotting time of all was found to be in normal range (3-5 minutes). When aqueous extract of *Ocimum sanctum* and *Zingiber officinale* was mixed in the blood samples (0.5 ml) in different ratios (1:0, 0:1, 1:1, 1:2 and 2:1), it significantly showed prolongation in the clotting time in the following manner.

BLOOD GROUP	AVERAGE CLOTTING TIME OF BLOOD SAMPLES (in sec)	CLOTTING TIME OF BLOOD SAMPLE WITH DRUG (OCIMUM SANCTUM AND ZINGGIBER OFFICINALE) IN DIFFERENT RATIO (in sec)				
		1:0 O. Sanctum (300 µl)	0:1 Z. Officinale (300 µl)	1:1 O. Sanctum (150 µl) Z. Officinale (150 µl)	1:2 O. Sanctum (100 µl) Z. Officinale (200 µl)	2:1 O. Sanctum (200 µl) Z. Officinale (100 µl)
O +ve	235	530	615	670	825	710
A +ve	220	465	595	635	760	680
B +ve	245	475	605	645	770	660
AB +ve	270	490	605	655	805	725

Table 1: CLOTTING TIME OF BLOOD SAMPLE WITH DRUG (OCIMUM SANCTUM AND ZINGGIBER OFFICINALE) IN DIFFERENT RATIO



GRAPH 1: CLOTING TIME OF BLOOD SAMPLE WITH DRUG (OCIMUM SANCTUM AND ZINGGIBER OFFICINALE) IN DIFFERENT RATIO

DISCUSSION

Anticoagulant such as warfarin is the drug of choice to treat thrombosis but according to meta-analysis there are various adverse effects associated with warfarin is bleeding. Apart from that, the newer anticoagulant drugs such as apixaban and edoxaban which have wider therapeutic windows do make the laboratory monitoring more difficult. In addition, these newer drugs also have uncertainty of drugs dosing in some patient’s populations such as patient with underlying chronic diseases. Hence, herbal medicines are widely used throughout the world as an alternative therapy to conventional anticoagulant. Besides, herbal medicines are cost effective and have fewer complications as compared to conventional anticoagulants⁷.

In this study, the synergistic effect of *O. sanctum* (Tulsi) and *Z. officinale* (ginger) aqueous extract on clotting time. Coagulation activity of *O. sanctum* and *Z. officinale* was measured by capillary glass method on blood samples collected from regular blood donors. The blood was tested against different ratio of aqueous extract of *O. sanctum* and *Z. officinale* as follows: 0:0, 0:1, 1:0, 1:1, 1:2, 2:1.

When *Ocimum sanctum* and *Zingiber officinale* was taken in 1:2 ratio the anticoagulant effect was found to be best in respect to other ratios (1:0, 0:1, 1:1, and 2:1).

CONCLUSION

The clotting time of blood increases when aqueous extract of *Ocimum sanctum* and *Zingiber officinale* was mixed with blood sample. Both the drug has its anticoagulant property as well.

In combination it gives synergistic activity. The anticoagulant property of ginger is comparatively more than holy basil. When *Ocimum sanctum* and *Zingiber officinale* was taken in 1:2 ratio the anticoagulant effect was found to be best in respect to other ratios (1:0, 0:1, 1:1, and 2:1). The following research work can be found useful for the future study of herbal anticoagulation therapy.

In this study, we have found that the aqueous extract of *O. sanctum* showed anticoagulation effects in human plasma by prolongation of clotting time. This finding led to the conclusion that *O. sanctum* leaves and *Z. officinale* rhizomes may act as an anticoagulant and thus, potentially may replace the current conventional anticoagulant drugs. Despite that, further study is needed to look at which clotting factors are affected the most by *O. sanctum* leaf extract and *Z. officinale* rhizomes extract in combination; either the intrinsic clotting factors, the extrinsic clotting factors or the common pathway coagulation factors.

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