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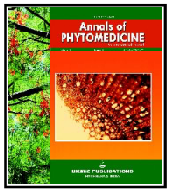

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Anticoagulation effect of extracts of leaves of *Ocimum basilicum* L. and *Ficus palmata* F.

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Abstract

Ocimum basilicum L. and *Ficus palmata* F. are two famous plants, found in Saudi Arabia and are part of food and beverages from ancient time. In this study, we evaluated the effect of leaf and fruit extracts of *O. basilicum* and *F. palmata* on prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT). The aqueous, methanol, and ethanol extracts of leaves and fruits were prepared to a concentration of 50 mg/ml. PT and APTT were measured using an automated digital coagulation analyser using normal and poor plasma platelet samples. The plasma samples were tested against different concentrations of both plant extracts as: 0.1, 0.5, 1.0 mg/ml. The clotting and bleeding time were determined by anticoagulation activity *in vitro*. The results show that the aqueous leaf extract of *O. basilicum* and *F. palmata* prolongs the APTT, TT, and PTT significantly. The methanol and ethanol extracts of fruits of *F. palmata* produce significant change in PTT, APTT (p<0.05) values but no significant effect on TT values in relation to control. The results show that leaf extracts of *O. basilicum* and *F. palmata* produce significant effect on coagulation activity of human serum, so both species can be potentially drugs targets of naturally derived anticoagulants.

1. Introduction

Herbs and medicinal plants are part of daily life in Asian and Middle East countries. The use of medicinal plants is considered effective and safe due to their natural occurrence from ancient time (Farouk *et al.*, 2016). Medicinal plants are big source of pharmaceutical drugs around the world and due to their diversity of compounds with minimum side effects (Aly *et al.*, 2014). These phytochemicals contain many bioactive compounds like flavonoids, alkaloids, terpenoids, steroids, phenols, and tannins (Said *et al.*, 2010; Rasool *et al.*, 2017; Awuchi *et al.*, 2021). The herbal medicines prepared from different plants like *Allium sativam*, *Melicope semecarpifolia*, and *Ocimum sanctum* are recorded to exhibit anticlotting activities (Almarshad, 2019; Appiah *et al.*, 2020).

Many *Ocimum* sp. are also found effective in haemostatic and cardiovascular disease conditions. In countries like India, species of *Ocimun* are considered holy basil and worshiped (Opalchenova *et al.*, 2003). Similarly, *Ficus* sp. is also found to be effective against 50 different diseases (Patel *et al.*, 2014). *Ficus* sp. is also reported beneficial against liver diseases, asthma, gastrointestinal diseases, and many skin diseases. Phytochemical study of dry leaves of *Ficus* sp. recorded phytosterols, amino acids, hydrocarbons, volatile components, phenolic compounds, fatty acids, and many other metabolites. Some other commonly studied plants are angelic root, devils claw, ginseng, alfalfa, and garlic. In search of new potential drugs which are more powerful, easily affordable, and safe. We conducted this study to evaluate the haemostatic effect of aqueous, methanol, and ethanol extracts of *O. basilicum* and *F. palmata*.

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2. Materials and Methods

2.1 Plant material collection

The leaves and fruits of *O. basilicum* (Specimen No. Linn749.5) and *F. palmata* (Specimen No. Foss 3418483) were collected from residential area around Majmaah. The botanical identification was conducted by Botany Department of Gauhati University, Assam. Methanol and diethyl ether were supplied by Loba chemicals, India. After collection, the plant parts were dried in shade and grounded to fine powder (Prabhu *et al.*, 2019).

2.2 Extract preparation

The aqueous plant extracts were prepared by soaking 10 gm of plant extract powder in 100 ml sterile boiled water for a week at 25°C with interim shaking. After one week, the mixture is then centrifuged at 5000 rpm for 5 min and the supernatant is then evaporated by rotary evaporator. The methanol and ethanol extract of plant species are obtained by dissolving 10 gm of powder in 100 ml of 70% methanol and ethanol for 1 week, followed by centrifugation and evaporation of extract.

2.3 Blood sample collection and preparation

The selection of volunteers was based on health status with no disease history, no medication, and not doing smoking. The ethical approval for recruitment and blood sample collection is taken from Gauhati University, Assam, India under Number: - 64/29952.The blood samples were collected in citrate tube, and each sample was

prepared by centrifugation of sample at 3000 rpm for 15 min to get platelets poor plasma (PPP). Within 2 h of sample collection the PT, APTT, and TT were analysed.

2.4 Whole blood bleeding and coagulation time

Blood coagulation time was assessed by following modified method of Lee and White (Lee and White, 1913). In this method, 1 ml of blood sample was placed in a standard dry test tube at 37°C in water bath. The coagulation time of each test tube was measured at each 30 min time interval until the test tube can be inverted without blood spilling. The coagulation time is measured and an average is taken for each test. To record the effect of plant extracts on coagulation time, each plant extract is added in 3 different concentrations of 0.05 ml, 0.1 ml, and 0.2 ml which is equitant to (21, 42, and 84 mg/ml) to each blood sample and clotting time is recorded.

To measure the effect of plant extracts on bleeding time, thumb prick blood was absorbed lightly by touching it on filter paper until bleeding stops and no more strain is obtained on filter paper. The effects of extract were measured by adding the plant extract immediately to the pricked thumb blood and time of blood stopping is measured.

2.5 Measurement of PT, APTT and TT

The *in vitro* assay of PT was carried out by incubating 50 ml of PPP with 50 ml of each plant extract for 5 min at 37°C. The clotting time was immediately recorded after adding 100 ml PT reagents. The APTT was measured by adding 50 ml of PPP to 50 ml different plant extract and incubated for 2 min at 37°C, followed by adding 50 ml APTT reagent and incubated at 37°C for another 3 min. The APTT clotting time was recorded immediately after adding 100 ml of $CaCl_2$ (Calcium chloride). The thrombin time is calculated by Hardisty and Ingram method (Hardisty *et al.*, 1956). 0.05 ml of ethanol, methanol, and aqueous extract of *O. basilicum* and *F. palmata* was added to 0.1 ml plasma in a normal tube, followed by 0.2 ml of thrombin solution and time of fibrin clot was recorded. The control for all tests was prepared by replacing plant extract with 0.05 ml of normal saline.

2.6 Statistical analysis

The data obtained is presented as mean ± SEM. The statistical analysis of the data was done using Prism software version 8.2 (San Diego, CA, USA). The results of control and plant extract treated groups were analysed using one-way ANOVA and $p<0.05$ was considered significant.

3. Results

The result of examined samples were denoting samples have no individual variation ($p<0.01$) in data for TT, PT, APTT, blood coagulation, and bleeding time. Data of various result shows that leaf extract of both *O. basilicum* and *F. palmata* significantly increases PT, APTT, and TT time.

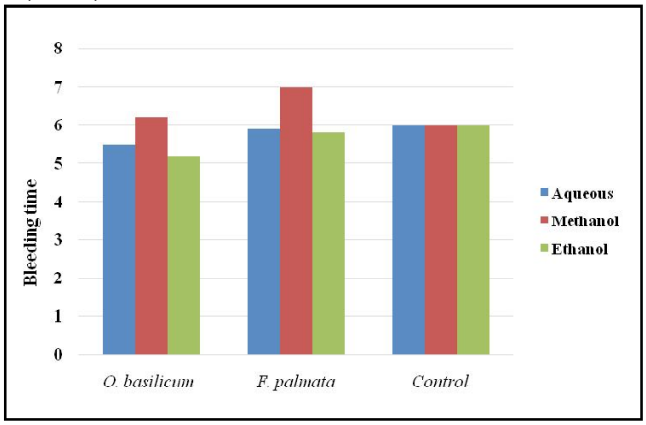

Figure 1: Effect of aqueous, methanol, and ethanol extract on bleeding time in comparison to control which does not have any extract added.

Table 1: PT, APTT, and TT assay (*O. basilicum* and *F. palmata*) of both plant extracts

Plant species	Type of extract	PT (0.05)	PT (0.1)	PT (0.2)	*p* values	APTT (0.05)	APTT (0.1)	APTT (0.2)	*p* values	TT (0.05)	TT (0.1)	TT (0.2)	*p* values
O. basilicum	Aqueous	170.8	168.9	171.6	0.002*	356.9	362.2	345.6	0.001*	11.5	10.5	11.7	0.003*
	Methanol	15.9	14.8	14.2	0.785	50.6	79.4	64.3	0.002*	21.7	15.2	12.1	0.001*
	Ethanol	34	32	34	0.003*	355.4	376.5	243.2	0.002*	10.3	11.2	11.6	0.001*
F. palmata	Aqueous	110.7	115.8	112.4	0.001*	320.8	354.3	322.5	0.001*	14.1	15.6	12.1	0.002*
	Methanol	11.9	16.5	14.4	0.889	82.4	87.2	44.2	0.445	16.4	17.5	14.3	0.001*
	Ethanol	33	34	24.5	0.004*	129.3	112.6	234.4	0.185	12.1	11.2	10.8	0.001*

*$p<0.05$ represent level of significance.

3.1 Thrombin time

The results of thrombin time of the study showed 0.05 ml and 0.1 ml aqueous extract of both plants *O. basilicum* and *F. palmata* produced very significant decrease in TT time in comparison to control (Table 1).

3.2 Prothrombin time and activated partial thromboplastin time

Ethanol and aqueous extract of *O. basilicum* prolong PT and APTT significantly ($p<0.05$) whereas, the aqueous leaf extract of *F. palmata* very significantly increases PT and APTT values but their was no effect on TT values (Table 1).

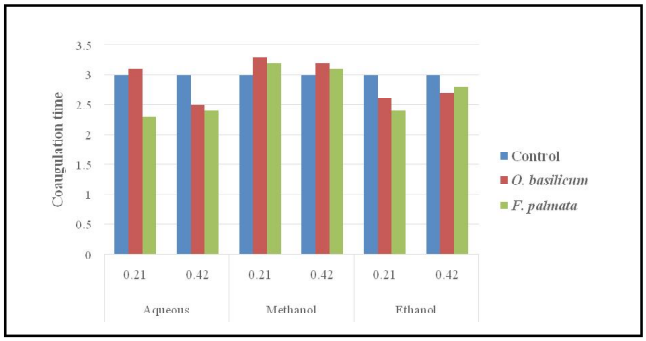

Figure 2: Effect of aqueous, methanol, and ethanol extract on coagulation time in comparison to control.

3.3 Blood clotting and bleeding time

The aqueous and ethanol extract of both the plants in 21 and 42 mg/ml concentrations produced a significant decrease with a *p* value of 0.023. Ethanol and aqueous extract of both the plants reduced the bleeding time significantly in comparison to control (Figure 1). The plasma clotting time was measured with different concentrations of (0.05 ml, 0.1 ml, and 0.2 ml) of both plant extracts. The aqueous, methanol and ethanol extract of *O. basilicum* in 21 and 42 mg/ml reduced plasma clotting time as calculated in minutes (Figure 2).

4. Discussion

O. basilicum also known as sweet basil is used as an aromatic and medicinal plant and also added to food and beverages for flavour. Similarly, *F. palmata* is a common fruit known for its lots of medicinal benefits. Many studies are conducted to study different phytochemical structures and medicinal properties of these plants including antimicrobial and antioxidant activities. Due to broad use of these two plant species, we selected them for our study as no similar study was conducted on these two plants.

The analysis of PT, TT, and APTT are important to screen the haemostatic functions of a patient having bleeding related disorders. Even though these are not considered specific tests but a lot of research are conducted in search of drugs which can help in reducing time for PT, TT, APTT, bleeding, and clotting time (Abdallah *et al*., 2019). The result of our studies indicate reduction in PT and APTT which is very important to fall within the normal ranges as prolonged PT, TT, and APTT can be due to deficiency in one or more than one factors II, V, VIII, IX, X, XI, and XII. Similarly, short PT is found in cancer and hypercoagulable due to which, the surgery becomes difficult. Similar data was recorded with aqueous and ethanol extract of *T. spicata* and *S. thymbra* (Emeka, 2021). The methanol extract of both the plants did not produce any significant effect on PT, TT, and APTT ($p<0.05$). Different studies conducted to find out the effect of extracts of *T. spicata*, *S. thymbra* and *V. fruticulosum* also recorded significant increase in PT and APTT values but, the results were not very highly significant (Farouk *et al.*, 2016; Hamid *et al.*, 2020). The normal range of APTT is 34 sec, plant extracts of *O. basilicum* and *F. palmata* extract reduced the time 30.4 ± 0.50 and 6.9 ± 0.30 sec in 0.05 ml, and 0.1 ml concentration. The aqueous and ethanol extract of *F. palmata* in 0.1 and 0.2 ml concentration reduce clotting time to a very significant time ($p<0.05$). Both extracts have great coagulation properties, which is independent of any activating agents.

Since the coagulation process in blood needs production of thrombin and conversion of fibrinogen to fibrin in presence of zymogens, and this occurs on activation of factor X. Single molecule of factor X can generate thousand thrombin molecules, making it a drug target for development of anticoagulation drugs. Different studies conducted of factor X confirms the effects of plant polyphenols on factor X and the study by Balanescu and his team recorded the presence of flavonoids like quercetin and rutin in *O. basilicum* (Balanescu *et al.*,2020). Our result confirms the effect of *O. basilicum*flavonoids on factor X and on PT and APTT. Our study of these plants presents them as good candidates for anticoagulation formulation development and drug discovery research.

5. Conclusion

In this study, we focused on efficiency of *O. basilicum* and *F. palmata*. The result clearly indicates that the leaf extract of these plants are potential drug candidates to be used to decrease bleeding time and help in coagulation process. This study also supports the synergic effect of bioactivities of different plants as anticoagulants to different degrees of solubility of different molecules. Since various factors like location, environment, period of collection and vegetative cycle can affect the composition further *in vivo* analysis of purified bioactive compounds is recommended.

Conflict of interest

The author declares no conflicts of interest relevant to this article.

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