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Phytochemical evaluation and *In-vitro* thrombolytic activity of hydro alcoholic extract of *Syzygium malaccense* leaves

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Abstract
The present study was designed to investigate preliminary phytochemical screening & in vitro thrombolytic activity of leaves of *Syzygium malaccense* belongs to family Myrtaceae. Prepared hydro alcoholic extracts of *Syzygium malaccense* showed presence of phytoconstituents like saponins, flavonoids, tannins, steroids and glycosides etc. Total flavonoid content in prepared extract was found to be 375.42±0.79µg equivalent quercetin /gm of dried extract. The % clot lysis of hydro alcoholic extracts of *Syzygium malaccense* leaves, at various concentrations like 100, 200, 300, 400, 500 µg/mL were found to be 20.15%, 23.64%, 32.12%, 55.21% and 40.65% respectively. In positive control, Streptokinase produced 71.43% clot lysis and in negative control, water produced 2.96% clot lysis. It was concluded that the in-vitro thrombolytic activity showed by the plants were due to the presence of phyoconstituents like tannins & flavonoids. Further studies are required to find out lead molecule as well as mechanism of action for this activity.

Keywords: *Syzygium malaccense*, flavanoids, thrombolytic action, % of clot lysis

1. Introduction
Thrombosis is the evolution of a 'thrombus' consisting of platelets, fibrin, red cells and white cells in the arterial or venous circulation. Failure of homeostasis leads to the evolution of blood clot in the circulatory vessels thus the resultant vascular blockage and in order to compensate the blockage it leads to serious consequences in atherothrombotic disease such as myocardial and cerebral infarction at time causing considerable mortality. Thrombosis is the principal disorder for myocardial infarction, stroke and pulmonary embolism. In the current clinical practices, the most commonly used anticoagulant agents include heparins, vitamin K-antagonists and their derivatives. These agents confer lesser benefit as compared to risk, to an extent of life-threatening side effects [1, 2].
Various berries due to their antioxidant power, anticancer and anti-inflammatory effects have attracted immense scientific interest. *Syzygium malaccense* (L.) belongs to the family of Myrtaceae and is an original plant from Malaysia, known as Malay apple [3]. It is also known as pomerac, mountain-apple or red-jambo. Various parts of the plant have been applied in traditional medicine, including seeds, bark, fruits and leaves as anti-inflammatory, antiviral, antifungal, antibacterial, antibiotic, remedy for itching, diuretic, as skin lotion and anti-edema [4]. The scientific literature reports antioxidant capacity of the edible part of red-jambo fruits, anti-inflammatory and antioxidant effects of leaves, cytotoxic power of leaves, and glycemia/cholesterolemia-lowering effects of the trunk bark [5]. Crushed leaves are used as antiemetic, purgative and also to treat bronchitis, tongue inflammation, dysentery, constipation, diabetes, cough, pulmonary cataract, headache and other ailments [6]. Since the plants are safer source of medicine and many plants are used for the treatment of thromboembolic diseases in various system of traditional medicine, this study is an attempt to enrich the knowledge of thrombolytic activity and phytochemical analysis of hydro alcoholic extracts of the leaf of *Syzygium malaccense* using in vitro thrombolytic activity [7, 8].

2. Material & Methods
2.1. Plant Collection & Identification
Fresh leaves of *Syzygium malaccense* were collected from medicinal plant garden of Pioneer Medical & Paramedical Campus, Ajwa road, Vadodara in the month of January 2018. The leaves were identified & authentified by botanist Dr. P K Patel, Head of Department, Sheth P.T Arts and Science College, Godhra, Gujarat bearing voucher specimen number

PPDC/COG/2018/001 as *Syzygium malaccense* belongs to the family Myrtaceae. The leaves were dried in shade at 25-30 °C for 7 days.

2.2. Preparation of Plant Extract
50 gm of coarsely powdered leaves of *Syzygium malaccense* were extracted with 200ml petroleum ether to remove fatty materials in a soxhlet apparatus for 24 hours. Extracts were filtered & marc obtained was dried & was again extracted with hydro alcoholic (methanol) solvent (30:70) for 48 hour using soxhlet apparatus. Hydro alcoholic extract were filtered, collected & evaporated up to dryness to obtain solid mass. The hydro alcoholic extract was kept in airtight container in a deep freezer, maintained at 4 °C until the time of further use.

2.3. Preliminary Phytochemical Screening
The extract was subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. The phytochemical tests of extract were carried out qualitatively for the presence of alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins and steroids by using the standard methods [9].

2.4. Estimation of Total Flavonoid Content
Total flavonoid content was measured by aluminium chloride colorimetric method. 0.5 mL of standard solution or test extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aqueous aluminum chloride, 0.1 ml of 10 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was incubated for 30 min at room temperature and the absorbance of the reaction mixture was taken at 415nm with a UV Spectrophotometer. Blank solution was prepared by substituting 10% aluminum chloride with the same amount of distilled water. The calibration curve of standard Quercetin (10-100 µg/ml in methanol) was prepared for comparison of test extracts [10].

2.5. Thrombolytic Activity
2.5.1. Preparation of Streptokinase
5 ml distilled water was added to the lyophilized SK vial of 15,00,000 I.U. and it was mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) and it was used for in vitro thrombolysis study.

2.5.2. Collection of blood
Blood was withdrawn from healthy human volunteers and 1 ml of blood was transferred to the previously weighted sterile eppendorf tubes and was allowed to form clot.

2.5.3. Determination of Thrombolytic activity [11, 12]
The eppendorf tubes were incubated at 37 °C for 45 minutes. Serum was completely removed after clot formation, without disturbing the clot and each eppendorf tubes having clot was again weighted to determine the clot weight (clot weight= weight of clot containing tube- weight of tube alone.) To each eppendorf tube containing pre-weighted clot, 100 µL of aqueous solutions of different extracts along with the crude extract was added separately. As a positive control, 100µL of streptokinase and a negative non thrombolytic control, 100µL of distilled water were separately added to the control tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and eppendorf tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis is shown below:

% clot lysis= (Weight of the lysis clot/ Weight of clot before lysis)*100

3. Results & Discussion
3.1. Phytochemical screening
Phytochemical screening was performed on the crude hydro alcoholic extract of *Syzygium malaccense* using standard procedures to identify the active phytoconstituents. It revealed the presence of saponins, flavonoids, tannins, steroids and glycosides. [Table:1]

Table 1: Preliminary phytochemical screening of hydro alcoholic Extracts of *Syzygium malaccense*

Class of Phytoconstituents	Result
Carbohydrates	Negative
Alkaloids	Negative
Flavonoids	Positive
Phenolics/Tannins	Positive
Proteins	Negative
Steroids	Positive
Glycoside	Positive
Saponins	Positive

3.2. Total flavonoid content
Total flavonoid contents of hydro alcoholic extracts of *Syzygium malaccense* extracts were found to be 375.42±0.79µg equivalent quercetin /gm of dried extract.

3.3. Thrombolytic activity
In case of in-vitro thrombolytic activity, addition of 100 µl Streptokinase, in positive control to the clots along with 90 minutes incubation at 37 °C, showed 71.54% clot lysis. In negative control, clots treated with 100 µl sterile distilled water has shown negligible (4%) of lysis of clot. The in-vitro thrombolytic activity study revealed that percent clot lysis of hydro alcoholic extracts of *Syzygium malaccense*, at various concentrations 100, 200, 300, 400, 500 µg/mL were 20.15%, 23.64%, 32.12%, 55.21% and 40.65% respectively [figure 1].

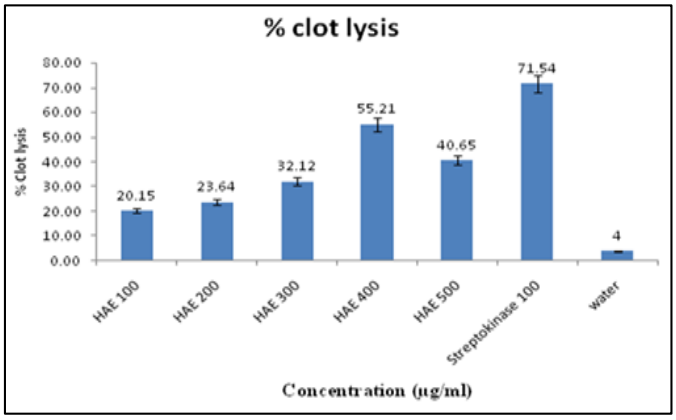

Fig 1: Effect of *hydro alcoholic extract of Syzygium malaccense on in-vitro* thrombolytic action

4. Conclusion
In above performed study it was reviled that hydro alcoholic extracts of *Syzygium malaccense* produced significant thrombolytic action comparable with streptokinase. Abundant amount of polyphenolics in the form of tannins and flavanoids might be responsible for said activity. Further studies can be performed using different fractions to identify a lead molecule

& thoroughly investigating the mechanism involved in said activity.

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