SHORT COMMUNICATION

Chemical characterization, thrombolytic and antioxidant activity of *Hibiscus tiliaceus* L. leaves

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**ABSTRACT**

The present study is aimed to evaluate in vitro thrombolytic activity and antioxidant activity of *Hibiscus tiliaceus* L. Chemical characterization of bioactive extract was determined by using GC-MS analysis. The thrombolytic potential was performed by the clot lysis method. Antioxidant activity was evaluated by an in vitro assay involving DPPH radical scavenging and hydrogen peroxide scavenging assay. Blood Clots when treated with sterile distilled water, petroleum ether extract, methanol extract and streptokinase showed 7.983 ± 2.197%, 34.10 ± 8.436%, 14.41 ± 5.276 and 62.21 ± 5.519 clot lysis, respectively. Petroleum ether extract shows significant activity when compared with control (P ≤ 0.05). All extracts showed concentration dependent free radical scavenging activity. Bioactive extract of *H. tiliaceus* contained 23 phytoconstituents as revealed by GC-MS study. The significant thrombolytic activity is exhibited due to presence of sterols as revealed by GC-MS analysis. Further study required to confirm in vivo clot lysis properties.

**ARTICLE HISTORY**

Received 9 November 2021
Accepted 5 March 2022

**KEYWORDS**

Antioxidant activity; GC-MS analysis; *Hibiscus tiliaceus*; thrombolytic activity

**1. Introduction**

Thrombolysis is the breakdown (lysis) of blood clots. Thrombolytic activate plasminogen to form plasmin, lead to fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator, the protein which activates plasmin (Longstaff 2018; Jilani and Siddiqui 2021). Thrombolysis cause by administering thrombolytic drugs that...
dissolve clots in blood. Generally streptokinase and urokinase used widely as thrombolytics. By dissolving the clot, the further complications are reduced. The anticoagulants like heparin prevent development of a clot and also reduces the clot size (Huang et al. 2020). Plants are natural sources of biomolecules and have a broad scope in drug discovery. Till today anticoagulants and antiplatelet agents from plant sources predominantly used in market (Ali et al. 2021).

*Hibiscus tiliaceus* L. is a tree (Malvaceae) also known as belpata, found throughout India. The extracts of Belpata leaves and flowers are used in the treatment of skin conditions, constipation, contraceptives, morning sickness, diabetics and fracture (Abdul-Awal et al. 2016; Chan 2016). Chemical constituents reported are gossypol, Gossypetin glycoside, Mansonones – D and F (Groupe et al. 2016), Hibiscones, Hibiscoquinones – A & D (Matsumoto et al. 2020), kampferol, Quercetin, Lapachol and p-coumaric acid (Nandagopalan et al. 2015) and triterpenoids (Chan 2016) in *H. tiliaceus*. The extract and phytoconstituents of *H. tiliaceus* have shown antimicrobial, analgesic, hypolipidemic, anti-inflammatory, antidepressant and antidiabetic activity (Kumar et al. 2009; 2010; Vanzella et al. 2012; Abdul-Awal et al. 2016). The phytoconstituents from *H. tiliaceus* were evaluated for antioxidant and a-glucosidase inhibitory activities recently (Vinh et al. 2021). From literature review it reveals that *H. tiliaceus* has traditionally used in blood disorder (CSIR 1959). However thrombolytic activity and antioxidant activity of various extracts of leaves of *H. tiliaceus* not been reported till. The objective of present study is to investigate petroleum ether extract and methanol extract of *H. tiliaceus* L. leaves for thrombolytic activity and antioxidant activity. The petroleum ether extract showed significant thrombolytic activity, so further study carried out to investigate phytoconstituents present in petroleum ether extract by GC-MS analysis.

2. Result and discussion

Several studies have been conducted to discover that plants and natural food sources, as well as their supplements, have antithrombotic (anticoagulant and antiplatelet) effects, and there is evidence that consuming such plant materials leads to the prevention of coronary events and stroke (Joshipura et al. 1999). The various thrombolytic agents available which was obtained by recombinant DNA technology but side effects associated to these drugs leads to complications. Platelets play a significant role in the development of atherothrombosis and also damage the regions of endothelial surface (Watt et al. 2012). We have used streptokinase as a positive control which is widely used thrombolytic agents that converts plasminogen to plasmin. The addition of streptokinase along with 90 minutes of incubation at 37 °C resulted in 62.21 ± 5.519% clot lysis. Clots when treated with sterile distilled water (negative control) showed only negligible clot lysis (7.983 ± 2.197%). Blood Clots when treated with petroleum ether extract and methanol extract showed 34.10 ± 8.436% and 14.41 ± 5.276% clot lysis respectively. A significant thrombolytic activity was observed on treatment the clots with petroleum ether extract of *H. tiliaceus* when compared with negative control (Table S1). Our findings may significantly demonstrated an application in cardiovascular health.
Free radicals are generated by the body’s endogenous system, exposure to toxicants or may induce different pathological conditions. Excess generation of free radicals causes cellular damage and promotes inflammation, thrombosis, aging, cancer and other related diseases. Antioxidants are aiding in decreasing and preventing damage from free radical reactions because of their capacity to donate electrons which deactivate the radicals. (Moura et al. 2015). DPPH is a nitrogen centered free radical with an odd electron which gives a prominent absorbance at 517 nm. The reduction in absorbance of DPPH radicals was caused by radical scavengers and was due to the scavenging of hydrogen donation (Dehpour et al. 2009). The different extracts of *Hibiscus tiliaceus* leaves have been studied earlier for antioxidant activity (Ramproshad et al. 2012; Samsudin et al. 2019; Vinh et al. 2021). In these studies, only DPPH method was used for evaluating antioxidant activity while we evaluated this activity by hydrogen peroxide assay as well. The findings in earlier studies have been confirmed by us through dual assay. Various concentrations of petroleum ether and methanol extracts of leaves of *H. tiliaceus* and ascorbic acid were found to be scavengers of DPPH radical and an IC₅₀ given in Table S2 (Figure S1a). H₂O₂ radical scavenging activity is a useful method for assessing the antioxidant properties of samples. H₂O₂ is usually nonreactive to cells but may give rise to hydroxyl radicals, which are toxic to cells. Therefore, removal of H₂O₂ is important and it can be carried by antioxidants which donate an electron to H₂O₂ and convert to water, thus blocking the production of hydroxyl radicals and protecting from cell injury. The potential of electron donation is directly proportional to its antioxidant capacity. Petroleum ether and methanol extracts of *H. tiliaceus* leaves and ascorbic acid were found to be scavengers of H₂O₂. An IC₅₀ value is given in table S2 (Figure S1b). The extracts of *H. tiliaceus* might be used to provide a good H₂O₂ scavenger for humans.

Phytochemical investigation of petroleum ether extract of *H. tiliaceus* leaves was carried out by GC-MS analysis (Figure S2) as it shown significant activity. GC-MS analysis reveals that it contains sterols, amide, esters, alkenes and so on. (Table S3). The Phthalic acid, hex-3-yl isobutyl ester and Phthalic acid, di(6-methylhept-2-yl) ester are phthalates which are improbable phytochemicals or artifacts (Bianco et al. 2014; Venditti 2020). Due to presence of steroids, leaves of *H. tiliaceus* may exhibited fibrinolytic activity (Hoffmann and Klöcking 1988; Lowe 1993). Our findings may have significant implications for cardiovascular health. In addition, this finding may indicate the possibility of developing novel thrombolytic compounds from *H. tiliaceus* leaves extracts. Further studies are required to isolate and characterize the compounds responsible for thrombolytic activity.

3. Experimental

Experimental details relating to this paper are given in Supplementary material.

4. Conclusion

On the basis of the findings of the present study, it is observed that *H. tiliaceus* L. shows remarkable thrombolytic activity due to presence of sterols. It also shows good
antioxidant activity. Therefore, steps should be taken to observe in vivo clot dissolving potential and isolation of phytoconstituents of these extracts.

**Acknowledgement**

Authors are thankful Dr. A. S. Dhake, Principal, S.M.B.T. College of Pharmacy, Dhamangaon, India for providing necessary facilities.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

The author(s) reported there is no funding associated with the work featured in this article.

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