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Research Article

Phytochemical, Fluorescence Analysis, *In vitro* Antioxidant and *In-vitro* Thrombolytic Activity of Kabasura Kudineer Herbal Concoction

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Abstract

Objective: The purpose of the study is to carry out fluorescence analysis, phytochemical, *in vitro* antioxidant *In-Vitro* Thrombolytic Activity of Kabasura Kudineer Herbal Concoction.

Methods: In the present study, fluorescence analysis, phytochemical, antioxidant *In-Vitro* Thrombolytic Activity of Kabasura Kudineer Herbal Concoction are carried out using standard procedures.

Result: The fluorescence analysis under visible and ultraviolet light for Kabasura Kudineer herbal concoction treated with various chemical reagents shown different fluorescence effect. It showed a significant antioxidant activity in DPPH, Reducing Power and H_2O_2 scavenging methods. From our study, we also found that it showed 41.6% clot lysis activity respectively and they showed significant % of clot lysis effect with reference of Streptokinase (75.2%) and water (2.93%).

Conclusion: The findings of the present study suggest that Kabasura Kudineer herbal concoction could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing has significant thrombolytic action. Additional studies are greatly essential for further drug development.

Keywords: Kabasura Kudineer herbal concoction; DPPH; H_2O_2 scavenging; Reducing Power and *In-Vitro* Thrombolytic Activity

Introduction

Traditional systems of medicine still be widely practiced on many accounts. Population rise, insufficient supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases increased the utilization of plant materials as medicines for an honest kind of human diseases. Kabasura kudineer choornam may be a polyherbal siddha formulation containing 15 ingredients recommended for effective controlling of common respiratory ailments like cold, cough, breathing difficulty and flu because the principal organ of kapha is lungs. Kaba indicates kapha dosha which recommends fever thanks to excess accumulation of kapha (mucus, phlegm) asura means herbs that alleviate the symptoms, kudineer denotes decoction and choornam indicates powder [1]. The tactic of preparation of kudineer is straightforward and therefore the phytoconstituents do not undergo any major change while processing and preparation, unlike other traditional formulations [2]. The preparation was reported to possess anti-inflammatory, antipyretic, antibacterial property [3] also to bind SARS-CoV-2 spike protein by *in silico* studies [4].

Thrombus (blood clot) can develop in blood cardiovascular system due to homeostasis, which causes vascular blockage, which will be responsible to health threatening results. Atherothrombotic diseases like cerebral or acute myocardial infarct also can even cause death [5]. Thrombolysis may be a complex mechanism, which interacts with clot components and surrounding plasma. During this interaction, plasmin, plasminogen, urokinase and fibrin are involved [6]. The thrombolytic activity of plasma is physiologically vital [7,8]. Usually, thrombolytic agents are anistreplase, streptokinase, alteplase, TPA (tissue plasminogen activator) and urokinase [9].

All available thrombolytic agents still have significant shortcomings, including the necessity for giant doses to be maximally effective, limited fibrin specificity and bleeding tendency, thanks to shortcoming of accessible thrombolytic drugs, studies are underway to style new improved recombinants variants regimen [10]. Aspirin and Heparin are considerably effective for activation of lysis and prevention of reclusion [11]. The selective antiplatelet agents and thrombin inhibitor are most powerful though safety is yet a key concern. The utilization of herbal for treatment of disease has been in practice since past. Herbal medicines are considered safer thanks to their natural activity [12]. It's been reported from studies that herbal products showing their thrombolytic activity significantly [13]. Within the present investigation, an effort has been made enrich the knowledge of phytochemical screening, fluorescence analysis, *in vitro* antioxidant analysis and thrombolytic activity of aqueous extract of Kabasura Kudineer herbal concoction.

Materials and Methods

Herbal preparation

Commercially available Kabasura Kudineer herbal concoction

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Table 1: Ingredients of Kabasura Kudineer Ayurvedic concoction.

Sl. No	Botanical Name	Plant part
1	*Zingiber officinale Rosc*	Rhizome
2	*Piper longum L.*	Fruit
3	*Syzygium aromaticum (L.) Merr & L. M. Perry*	Flower bud
4	*Tragiain volucrata L.*	Root
5	*Anacyclus pyrethrum (L.) Lag.*	Root
6	*Hygrophilaauriculata (Schum.) Heine*	Root
7	*Terminalia chebula Retz.*	Pericarp
8	*Justicia adhatoda L.*	Leaf
9	*Plectranthus amboinicus (Lour) Spreng*	Leaf
10	*Saussurea costus (Falc.) Lipsch.*	Root
11	*Tinospora sinensis (Lour) Merr.*	Stem
12	*Premnaherbacea Roxb. (Official substitute)*	Root
13	*Andrographis paniculata (Burm.f.) Nees*	Whole plant
14	*Cissampelos pareira L.*	Root
15	*Cyperus rotundus L.*	Rhizome

were purchased from Local market Tamandu, India (Table 1). The water solvent extraction procedure was used to prepare the extract. 100mg extract was suspended in 10ml distilled water and the suspension was shaken forcefully on a vortex mixer. The suspension was kept overnight and decanted to get rid of the soluble supernatant, which was filtered through a 0.22-micron syringe filter. 100µl of this aqueous preparation of herbs was added to the micro centrifuge tubes containing the clots to see thrombolytic activity.

Fluorescence analyses

These analyses were carried out as per the standard procedures [14]. In the present study, the powdered was treated with various chemical reagents like aqueous 1(N) sodium hydroxide, alcoholic 1(N) sodium hydroxide, 1(N) hydrochloric acid, 50% sulphuric acid and concentrated nitric acid, ferric chloride, etc. Then their extracts were subjected to fluorescence analysis in day light and UV light (254nm and 365nm).

DPPH radical scavenging assay

Accurately weighed 4.3mg of DPPH was dissolved in 3.3ml of methanol in a test tube [15]. Solution was protected from light by covering with aluminum foil. 150µl of above solution was taken and diluted up to 3ml with methanol, the absorbance of this solution was taken immediately at 516nm on UV spectrophotometer using methanol as blank. This reading was served as control reading. For the test and standard, the aliquots of various concentration ranging were prepared. For the assay 150µl of the test or standard solution was added to 150µl of DPPH solution and diluted up to 3ml with methanol, the absorbance of this solution was taken after 15 minutes at 516nm on UV spectrophotometer using methanol as blank.

The absorbance was taken in triplicate manner. The % Scavenging activity was found by using following formula:

% scavenging activity = {(absorbance at blank) – (absorbance at test)/(absorbance at blank)} X 100

Hydroxyl radical scavenging activity

The Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system [16]. The reaction mixture contained deoxy ribose (2-8mM), Fecl (0.1mM), EDTA (0.1mM), H_2O_2 (lmM), ascorbate (0.1mM), KH PO - KOH buffer (20mM, 24 pH 7.4) and various concentrations (25-400 ìm of extracts and std 10 to 80 ìm/ml) of standard drug in a final volume of 1ml. The reaction mixture was incubated for 1hr at 37°C; deoxyribose degradation was measured with spectrophotometer at 532nm. The percentage of hydroxyl radical scavenging activity was determined by the formula:

% scavenging activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

Reducing power

The reducing power of a compound is significant indicator of its potential antioxidant activity. Increased absorbance of the reaction mixture indicates increased reducing power. Various conc. of the extracts in 1ml of water were mixed with phosphate buffer (2.5ml, 0.2M pH 6.6) and 1% potassium ferricyanide (2.5ml). The combination was incubated at 50°C for 20min. Aliquots of trichloracetic acid (2.5ml, 10%) were added to the mixture, which was then centrifuged al 3000g for 10min. upper layer of solution (2.5ml) was mixed with water (2.5ml) and freshly prepared $FeCl_3$ solution (0.5ml, 0.1%). The absorbance was measured at 700nm [17].

% Reducing activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

Determination of Clot Lysis

Clot lysis approaches were administered as reported earlier [18]. 6mL blood drawn from the healthy volunteers was distributed in 10 different pre weighed sterile micro centrifuge tubes (0.5mL/tube) and incubated at 37°C for 45min. After clot formation, serum was completely removed without disturbing the clot and every tube having clot was again weighed to work out the clot weight (clot weight = weight of clot containing tube - weight of tube alone). 100µL of aqueous extracts of Kabasura Kudineer herbal concoction was added. As positive and negative controls, 100µL of SK and water, respectively, was added separately. All the tubes were then incubated at 37°C for 90min and observed for clot lysis. Released fluid was removed and tubes were again weighed to watch the difference in weight after clot disruption. Difference obtained in weight taken before and after clot, lysis was expressed as percentage of clot lysis.

Statistical analysis

All experiments were performed in triplicate. The data were analyzed using Microsoft Excel and are expressed as mean ± standard deviation (n = 3).

Results and Discussion

Preliminary phytochemical examination

The phytochemical screening of the plant revealed the presence of Carbohydrates, Glycosides, Alkaloids, Phenolics Flavonoids, Tannins, Triterpenoids, Saponins and Steroids. Proteins and amino acids was not present in the extract (Table 2).

Table 2:

Sl. No	Phytochemical constituents	Kabasura Kudineer herbal concoction
1	Carbohydrates	Positive
2	Glycosides	Positive
3	Alkaloids	Positive
4	Phenolics	Positive
5	Flavonoids	Positive
6	Tannins	Positive
7	Triterpenoids	Positive
8	Saponins	Positive
9	Steroids	Positive
10	Proteins and amino acids	Negative

Table 3:

S. No	Powder + Reagent	Visible light	UV light
1	1N HCl	Brown	Light green
2	1N NaOH	Brown	Green
3	1N NaOH + Methanol	Green	Pink
4	50% KOH	Brown	Brown
5	50% H_2SO_4	Green	Green
7	Conc. HNO_3	Orange	Pale green
8	Acetic Acid	Green	Pink
9	50% HNO_3	Orange	Green
10	Iodine Solution	Colorless	Light green

Fluorescence Analysis of Kabasura Kudineer herbal concoction

In the present study, the powder sample of Kabasura Kudineer herbal concoction. Exhibited a green colouration under day light, and black in long UV (365 nm) light. The colour appearance of the sample in different reagents was shown in Table 3.

Presence or absence of various phytoconstituents exhibits varied colour response when reacting with specific chemicals. The fluorescence is restricted for a specific compound. Several phytocompounds fluoresce when aptly illuminated with proper light. A non-fluorescent compound mixed with florescent impurities can also fluoresce. Therefore, fluorescence method is additionally considerably necessary to standardize a crude drug material physically [19].

DPPH radical scavenging activity

DPPH radical Scavenging assay within the present study several radical scavenging activities of Aqueous extract of Kabasura Kudineer herbal concoction were evaluated by DPPH scavenging assay. Aqueous extracts of PCC have gotten profound antioxidant activity. DPPH antioxidant assay is predicated on the power of DPPH, a stable radical, which gets decolorized within the presence of antioxidants [20]. The DPPH radical contains an odd electron, which is liable for the absorbance at 517nm and for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, it gets decolorized which may be quantitatively measured from the changes in absorbance at 517nm. The Aqueous extracts of Kabasura

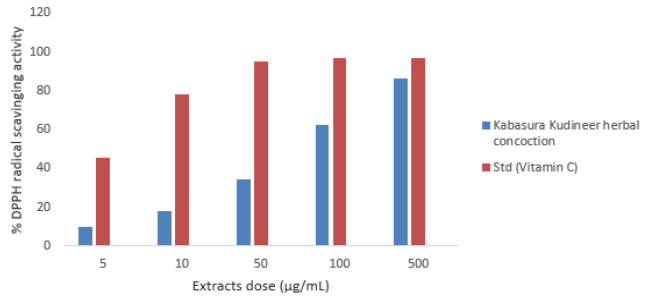

Figure 1: DPPH radical scavenging activity of varying concentrations of Kabasura Kudineer herbal concoction.

Kudineer herbal concoction exhibited a big dose dependent inhibition of DPPH activity. During this study, at 500µg/ml, the Kabasura Kudineer herbal concoction extract showed highest inhibition of DPPH activity shown in (Figure 1). The results of DPPH-free radical scavenging assay suggest that the Kabasura Kudineer herbal concoction extract is more capable of scavenging free radicals.

Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical created in biological system. It has been implicated as a major active oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions, which is capable of damaging almost every molecule found in living system causing lipid peroxidation and biological damage [21].

The maximum Hydroxyl radical scavenging effect was found at 500µg/ml concentration. The Kabasura Kudineer herbal concoction showed higher scavenging activity shown in Figure 2. This ability of the extracts shows the quenching ability of hydroxyl radicals, which seems to be an honest scavenger, of active oxygen species thus reducing the speed of chain reaction.

Reducing power activity

It is an honest indicator of antioxidant activity. The plant having high reducing power generally reported to hold high antioxidant potential too. Reduction of Fe (III) by electron-donating activity of the compounds reflects the possible antioxidant mechanism of the compound [22]. During this experiment, Ferric ions are reduced to ferrous ions with the colour of the reaction mixture changes from yellow to bluish green. The results for ferric reducing power activity of Kabasura Kudineer herbal concoction extract with compared to vitamin C are reported in Figure 3. Extract exhibited dose dependent reducing power potential. However, the efficacy was found to be less

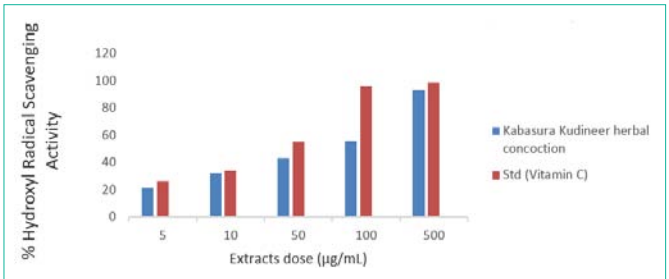

Figure 2: Hydroxyl radical scavenging activity of varying concentrations of Kabasura Kudineer herbal concoction.

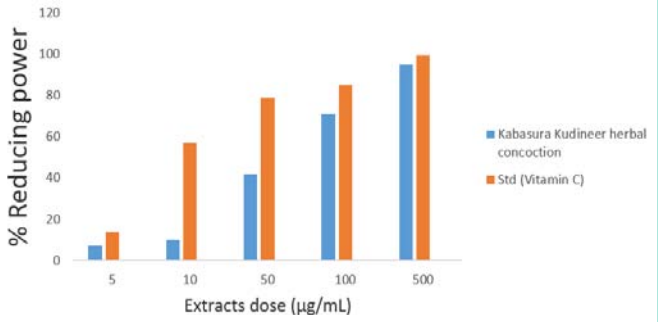

Figure 3: Reducing Power activity of varying concentrations of Kabasura Kudineer herbal concoction.

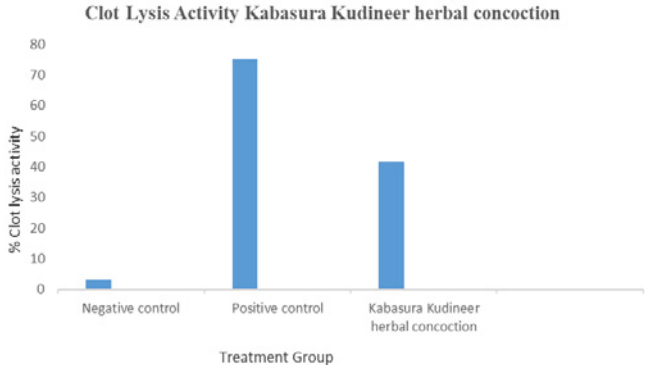

Figure 4: Comparative % *in vitro* thrombolytic effect of Kabasura Kudineer herbal concoction streptokinase and water (negative control).

than that of vitamin C.

Thrombolytic activity

In the current study of evaluation of the thrombolytic activity of Kabasura Kudineer herbal concoction, with the comparison of the percentage of clot lysis of positive control (SK) to the negative control (DW), it had been evident that the dissolution of the clot was negligible upon addition of DW. The extract showed 41.6% of clot lysis, addition of 100µl Streptokinase has 75.2% of clot lysis, which is taken as positive control whereas distilled water which was taken as negative control showed negligible clot lysis of 2.93% which was incubated for 90 minutes at 37°C. The percentage of clot lysis between positive and negative control was found to be significant statistically.

The *in vitro* thrombolytic activity study revealed aqueous extract of Kabasura Kudineer herbal concoction, exhibits significant thrombolytic activity when compared with negative control. A malfunction of hemostasis and consequent formation of blood clots in the circulatory system can produce severe consequences such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and streptokinase [23]. A number of studies have been showed by several researchers to find out the herbs and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke [24-26]. In our present *in vitro* preliminary clot lysis test confirmed that, aqueous extracts of Kabasura Kudineer herbal concoction showed the thrombolytic activity. The maximum clot lysis activity was mostly observed in extract that means water-soluble compounds are mainly responsible for the thrombolytic activity. In addition, this finding may indicate the prospect of developing novel thrombolytic compounds (Figure 4).

Conclusion

From preliminary phytochemical evaluation it has revealed that this plant extracts contained several chemical constituents and these kinds of chemical constituents directly responsible for biological effects. In our present *in vitro* preliminary clot lysis test confirmed that extracts of Kabasura Kudineer showed the thrombolytic activity. Which suggests that Aqueous soluble compounds are mainly responsible for the thrombolytic activity. Additionally, this finding may indicate the likelihood of developing novel thrombolytic compounds. Further studies are needed to quantify the number of chemical constituents present during this plant extract and further studies help to isolate, characterize the compounds responsible for thrombolytic activity. In near future it is getting to be implemented as a thrombolytic for the event of patients suffering from atherothrombotic diseases.

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