Research Article

A Study on Thrombolytic, Cytotoxic and Antioxidant Potential of *Murraya Koenigii*

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ABSTRACT

The plant *Murraya koenigii* was selected and analyzed for thrombolytic, cytotoxic and antioxidant potential. Phytochemical analysis was performed in both aqueous and ethanolic extracts of *Murraya koenigii* to detect several kinds of phytoconstituents namely carbohydrates, proteins, phenols, steroids, saponins, quinones, alkaloids, flavonoids, tannins and volatile oils. The thrombolytic activity of the aqueous extract of the plant was found to be 26.17%. Correlation was carried out between serum cholesterol level and thrombolytic activity of *Murraya koenigii*, where no correlation was obtained. The cytotoxicity of the plant was determined using brine shrimp lethality assay which expressed a LC50 value of 6.46.Total antioxidant level in terms of Gallic acid equivalence was found to be 350.81±0.99 mg/g. Thus the present findings suggest that, the plant has thrombolytic, cytotoxic and antioxidant properties which could be explored for the treatment of cardiovascular diseases.

Keywords: *Murraya koenigii,* thrombolysis, cytotoxic, antioxidant effect.

INTRODUCTION

Herbal medicines form an important part of health care system in India because they are easily available, cheaper and safer than synthetic drugs[1]. Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines. Thus, natural products with pharmacological or biological activities still play a very important role in medicine[2]. Thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of morbidity and mortality in developed countries. Thrombolytic therapy uses drugs called thrombolytic agents, such as alteplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots[3].

Brine shrimp larvae have been used as a bioassay for a variety of toxic substances. The method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds[4]. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases such as cancer, atherosclerosis, diabetes, inflammation and ageing. Recently, many antioxidants have been isolated from different plant materials[5]. Curry leaf tree (*Murraya koenigii* L., Family: Rutaceae) is a plant which has various important uses in the traditional system of medicine in Eastern Asia. Based on ethno medicine, *Murraya koenigii* is used as a stimulant, antidysentric and for the management of Diabetes Mellitus. The plant is highly valued for its leaves an important ingredient in an Indian cuisine to promote appetite and digestion[6].

Therefore, the present study has been performed to evaluate *Murraya koenigii* for its thrombolytic, cytotoxic and antioxidant properties.

MATERIALS AND METHODS

Preparation of the plant extract

Fresh leaves of the plant were collected, washed and homogenized using water for the preparation of aqueous extract and the Organic extract was prepared using petroleum ether. These extracts were used further for the determination of thrombolytic, cytotoxic and antioxidant properties.

Phytochemical analysis of *Murraya koenigii*

Qualitative analysis was carried out with fresh leaves of the selected plant to detect the phytochemical constituents.

Assessment of thrombolytic activity

Thrombolytic activity of *Murraya koenigii* was determined using human blood [7].

Determination of total cholesterol

Total cholesterol was estimated in serum obtained from the human blood samples used for thrombolytic activity following (CHOD-PAP) kit procedure[8].

Screening of cytotoxicity of plant samples

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extract of *Murraya koenigii*[9].

Determination of total antioxidant activity

The method was followed to determine the total antioxidant activity of the selected plant[10].

Statistical analysis

The significance between clot lysis by clavix and the plant extract by means of weight difference was tested by one

way ANOVA. Serum cholesterol and clot lysis were compared by correlation analysis. LC50 values for brine shrimp lethality bioassay were calculated by probit analysis. Experimental results of total antioxidant activity were expressed as mean ± S.D.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemical constituents

Phytochemical screening of the plant to determine the presence or absence of bioactive compounds was performed in this study. The results are tabulated in table 1.

Table 1: Phytochemical constituents of *Murraya koenigii*

Phytochemicals	*Murraya koenigii*	
	Aqueous	Ethanol
Carbohydrates	+	+
Aminoacids and proteins	+	+
Phenols	+	+
Sterol and steroids	+	+
Saponins	+	+
Quinones	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Volatile oil	+	+

'+'→Presence

In the present study phytochemical analysis was done to detect the active components in the plant extracts. Phytoconstituents namely carbohydrates, proteins, phenols, steroids, saponins, quinones, alkaloids, flavonoids, tannins and volatile oils were present in both aqueous and ethanolic extracts of *Murraya koenigii.*

These results are in agreement with [11] who indicated that the phytochemical screening of crude extracts of *Vitex leucoxylon* revealed the presence of alkaloids, flavonoids, terpenoids, steroids, phenolics, carbohydrates, amino acids, and quinones.

Thrombolytic activity of *Murraya koenigii*

Thrombolytic activity of aqueous extract of *Murraya koenigii* was determined using normal human blood with a series of concentration from 20 to 100 mg/ml and the percent clot lysis was determined. The values are represented in figure 1.

Clavix used as a positive control was incubated at 37ºC for 90 minutes along with the clot and it exhibited 58.38% clot lysis. The aqueous extract of *Murraya koenigii* showed a proportional increase in clot lysis with the increase in sample concentration and it exhibited 26.17% lysis for a maximal concentration of 100mg/ml. Water used as negative control exhibited minimum % of clot lysis (11.45). These findings are in agreement with previous study,[7] who state that the percent clot lysis of the *Fagonia Arabica* plant extract was significantly higher when compared to the negative control (water).

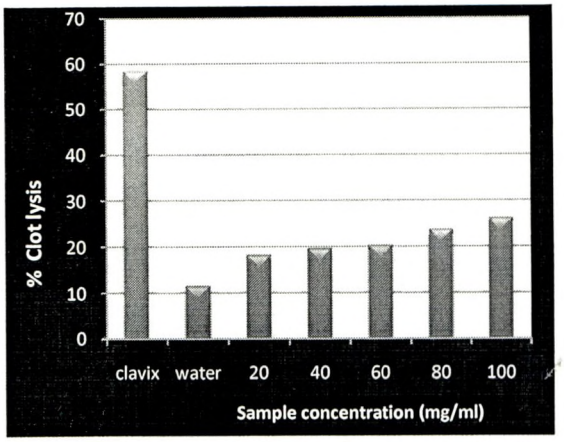

Figure 1: Thrombolytic activity of *Murraya koenigii*

Comparison of serum cholesterol level and % clot lysis of the plant extract

Total cholesterol was estimated in serum obtained from blood samples used for clot lysis. A comparison was made between serum cholesterol and percent clot lysis. The values are indicated in figure 2.

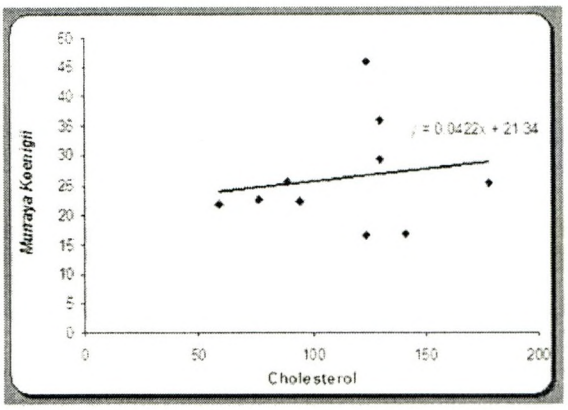

Figure 2: Comparison of serum cholesterol level and % Clot lysis of *Murraya koenigii* [R = 0.0265^{ns}; ns – Not significant at 5%]

Total serum cholesterol level and thrombolytic activity of the plant extract was compared using correlation analysis. The findings revealed that there is no correlation between serum cholesterol and % clot lysis.

Biosafety screening

Brine shrimp lethality assay was carried out to determine the cytotoxic effect of plant extract. The lethality of aqueous extract of *Murraya koenigii* to the brine shrimp (*Artemia salina)* was determined after 24 hours of exposure and the findings are indicated in figure 3.

The result of Brine shrimp assay was expressed in percentage lethality. The percentage mortality increased with increase in concentration of aqueous extract of *Murraya koenigii* and showed a significant cytotoxic

effect against brine shrimp nauplii with an LC50 value of 6.46 mg/ml. This finding is in agreement with [12] who reported that the bioassay with methanolic extract of *Tridax procumbens* and *Vernonia cinerea* showed better inhibition against brine shrimp.

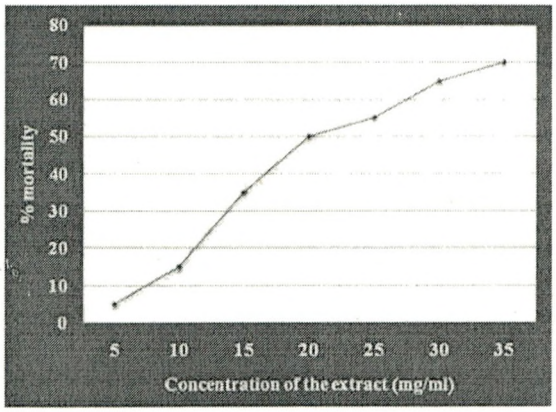

Figure 3: Cytotoxic effect of *Murraya koenigii*

Total antioxidant activity

The total antioxidant activity in terms of Gallic acid equivalence (mg/g) was calculated and the results are represented in figure 4.

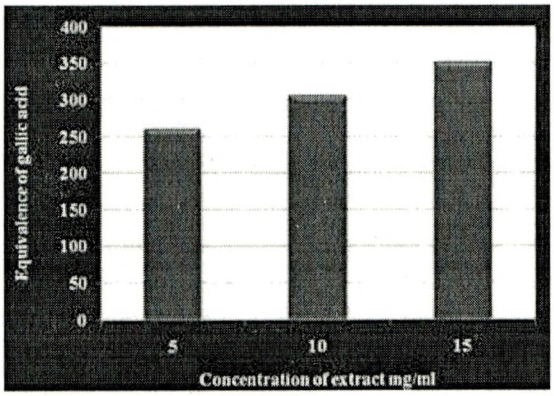

Figure 4: Total antioxidant activity of *Murraya koenigii*

Determination of the total antioxidant activity of the aqueous extract revealed an increasing trend with increase in concentration and the range was found to be 260.0 and 350.81mg/g. The above results are in agreement with old literature,[13] who found that Iranian *Ocimum* accessions have strong natural antioxidant properties and *Mentha piperita* has higher antioxidant activity.

CONCLUSION

Plants which possess medicinal properties are being exploited for their use in herbal preparations. The findings revealed that, *Murraya koenigii*, the plant chosen for the present study has antioxidative, thrombolytic and cytotoxic potential and can be further explored for their potent use in pharma industries.

REFERENCES

1. Acharya,S., Sahu,A.R. and Satyajit., Mohanta,S.R., Free radical scavenging activity of Thalamus of Nymphacea stellata Willd, International Journal of Pharmacy and Pharmaceutical Sciences, 2(4), 2010, 61-63.
2. Onocha, P.A., Oloyede, G.K. and Afolabi, Q.O., Chemical composition, cytotoxicity and antioxidant activity of essential oils of *Acalypha hispida* flowers, Int. J. Pharmacol., 7, 2011, 144-148.
3. Khan, I.N., Sarker, M.I., Almamun, A., Mazumder, K., Bhuiya, M.A.M. and Mannan, A., Cytotoxic and Thrombolytic Activity of Ethanolic Extract of Zanthoxylum Budrunga (Fam: Rutaceae) Leaves, European Journal of Scientific Research., 2, 2011, 303-310.
4. Mannan, A., Kawser, M.J., Ahmed, A.M.A., Islam, N.N., Alam, S.M.M., Emon, M.A.E.K. and Gupta, S.D., Assessment of antibacterial, thrombolytic and cytotoxic potential of Cassia alata seed oil, Journal of Applied Pharmaceutical Science, 2011, 56-59.
5. Kalim, M.D., Bhattacharyya, D., Banerjee, A. and Chattopadhyay, S., Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine, BMC Complementary and Alternative Medicine, *10,* 2010, 77.
6. Bonde, S.D., Nemade, L.S., Patel, M.R. and Patel, A.A., *Murraya koenigii* (Curry leaf): Ethnobotany, Phytochemistry and Pharmacology - A Review, International Journal of Pharmaceutical and Phytopharmacological Research, 1(1), 2011, 23-27.
7. Sweta, P., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori,G.M. and Daginawala, H.F., Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis, BMC Complementary and Alternative Medicine, 2007, 7-36.
8. Allain, C.C., Poon, L., Chan, S.G., Richmond, W. and Fu, P., Enzymatic Determination of Total Serum Cholesterol, Clin. Chem., 1974, 20, 470.
9. Oladimeji, H.O., Nia, R. and Essien, E.E., *in vitro* anti-microbial and brine-shrimp lethality potential of the leaves and stem of *Calotropis procera* (Ait)., Afr. J. Biomed. Res., 9: 2006, 205-211.
10. Prieto, P., Pineda, M. and Aguilar, M., Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E., Anal. Biochem, 269, 1999, 337-341.
11. Cathrine, L. and Nagarajan, N.P., Preliminary phytochemical analysis and antibacterial activity of leaf extracts of vitex leucoxylon l.f, International journal of current pharmaceutical research, 3, 2011, 45-56.
12. Sikder, M.A., Siddique, A.B., Hossian, A.K.N.M., Miah, M.K.,Kaisar, M.A. and Rashid, M.A., Evaluation of Thrombolytic Activity of Four Bangladeshi Medicinal Plants, as a Possible Renewable Source for Thrombolytic Compounds, Journal of Pharmacy and Nutrition Sciences, 1, 2011, 4-8.
13. Javanmardi, J., Stushnoff, C., Locke, E. and Vivanco, J.M., Antioxidant activity and total phenolic content of Iranian Ocimum accessions, Food chemistry, 2003, 547-550.

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