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STUDY OF ANTI-SICKLING PROPERTY OF *TINOSPORA CARDIFOLIA*

ANJU BHANDARI[a1], SUNEETA PATRA[b] AND P.K. PATRA[c]

[a]Bhilai Mahila Mahavidyalaya, Bhilai, Chhattisgarh, India
[b]Govt.N.P.G. College of Science, Raipur, Chhattisgarh, India
[c]Pt.J.N.M.College , Raipur, Chhattisgarh, India

ABSTRACT

***Tinospora cardifolia* (Guduchi, Amrita) Miers ex Hook. F. & Thomas is a widely used in folk and Ayurvedic systems of medicines. *Tinospora cardifolia* (Giloy) is indigenous, common shrub occurred in the Himalayas, tropical areas of India, China and mostly found in the dense forest of Chhattisgarh. This plant has been used in the treatment of sickle cell anaemia due to the presence of bioactive molecules. The present study investigates the primary screening of bioactive molecules of aqueous extract of *Tinospora cardifolia* stems and invitro antisickling test (Sickling reversal test). Phytochemical screening revealed the presence of alkaloids, flavanoids, phenols, steroids & cardiac glycosides. On the basis of results obtained from the invitro antisickling test, it has been noticed that the aqueous extract of *Tinospora cardifolia* stem exhibited antisickling property therefore it can be therapeutically used in the treatment of sickle cell anaemia. Sickle cell disease is a point mutation disorder quite prevalent in the state of Chhattisgarh.**

KEYWORDS: *Tinospora cardifolia*, Phytochemical screening, Bioactive molecules, antisickling properties.

Systematic position of *Tinospora cardiofolia*

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Order	-	Ranunculales
Family	-	Menispermaceae
Genus	-	*Tinospora*
Species	-	*cardiofolia*

Tinospora cardifolia (Guduchi,Amrita) Miers ex Hook.F. & Thomas is a widely used in folk and Ayurvedic systems of medicines. *Tinospora cardifolia* (Giloy) is indigenous, common shrub occurred in the Himalayas, tropical areas of India,China and mostly found in the dense forest of Chhattisgarh. It is glabrous and deciduous climbing shrub (linas) belonging to the family Menispermaceae Aima RK, 2003; Vaidya DB, 1994. The stem of *T.cardifolia* is greyish brown-black colour and bitter in taste. This plant grows as woody climber also known as woody climber or linas .leaves are simple, alternate, cordate shape, reticulate venation and long petiole. Male flowers grow in cluster and female flower grow in solitary form Chopra R N *et al* 1956.

Tinospora cardiofolia

Stems and powder of *Tinospora cardiofolia*

In Sickle cell disease (SCD) red blood cells form an abnormal sickle or crescent shape haemoglobin. This genetic disease is due to a mutation in the β-globin gene in which the 17th nucleotide is changed from thymine to adenine resulting which the sixth amino acid in the β-globin chain of haemoglobin glutamic acid - a polar amino acid is replaced by valine - a non-polar amino acid. This mutation decreases the affinity of haemoglobin for oxygen. At low oxygen tension the mutant haemoglobin known as sickle haemoglobin, polymerize within the erythrocytes cause the change of their shape from their normal disc like form resembling a sickle (Mehanna, 2001). Antisickling bioactive components occur in herbal plants can be used for treatment of sickle

[1]**Corresponding author**

cell disease. It has been seen that tribal people in Chhattisgarh usually use plant extract for the treatment of sickle cell patients. Patient of SCD are relieved from clinical manifestation by these plant.

MATERIALS AND METHODS

Collection of Plant Material

The fresh stems of *T. cardiofolia* were collected from botanical garden of our college. The stems were cut into small pieces and air dried on shadow for two weeks .After drying, the stems were grinded into powered by using a grinder machine before being subjected to phytochemical screening.

Preparation of Aqueous Extract by Soxhlet Extraction Method

Crude plant extract was prepared by soxhlet extraction method. About 50 gm of powered plant material was packed into a thimble and extract with 500ml of distilled water .The process of extraction was continued till the solvent in siphon tube of an extractor became colourless. After that the extractor was taken in a beaker and kept on the water bath till all the solvent got evaporated. Dried extract was kept in refrigerator at 4*c for phytochemical analysis. (Yadav RNS; Agarwala Munin, 2011)

Preliminary Phytochemical Analysis

The extract was screened for the presence of bioactive compounds by using following standard methods. (Harbone JB, 1973; Sofowora A, 1993; Kokate CK *et al* 2007)

Test for alkaloids

1. Wagner test: - 2ml of extract was treated with a few drops Wagner's reagent (iodine potassium iodide solution). Alkaloids give reddish brown precipitate with Wagner's reagent.

2. Hager's test: - 2ml of extract was treated with a few drops of Hager's reagent (saturated solution of picric acid). Alkaloids give reddish brown precipitate with Hager's reagent.

3. Mayer's test: - 2ml of extract was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution) .Formation of a cream coloured precipitate indicates the presence of alkaloids.

Test for Flavonoids

1. Shinoda test: - Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet, crimson red or occasionally green to blue colour appeared after few minutes which indicated the presence of flavonoids.

2. Lead acetate test: - Small quantity of the extract was treated with a few drops of lead acetate solution. Formation of yellow colour or yellow creamy precipitate indicated the presence of flavonoids.

3. Zinc HCL test: - To the test solution add a mixture of zinc dust and conc. HCL acid . It gave red colour after few minutes.

4. Alkaline reagent test: - Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Phenols

1. Ferric chloride test: - About 0.5 gm of the dried powered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue –black colouration.

2. Lead acetate test: - Small quantity of the extract was treated with a few drops of lead acetate solution. Formation of yellow colour or yellow creamy precipitate indicated the presence of flavonoids.

Test for Saponins

1. Foam test:- Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously . The formation of stable foam was taken as an indication for the presence of saponins.

Test for Steroids

1. Salkowski test: - 5ml each of extract was mixed in 2ml of chloroform and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2. Sulfur powder test: - Small amount of sulphur powder was added to the test solution, it sinked at the bottom.

Test for Tannins

1. Bramer's test: - 1 ml of plant extract was added 1ml of 10 % alcoholic ferric chloride solution formation of blue or greenish colour.

2. Ferric chloride test:- About 0.5 gm of the dried powered samples was boiled in 20 ml of water in a test tube and then filtered . A few drops of 0.1% ferric chloride was added and was observed for brownish green or blue –black colouration.

3. Alkaline reagent test:- Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of tannins.

Test for Terpenoids

1. Salkowski test: - 5ml of each extract was mixed in 2ml of chloroform and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac Glycosides

1. Keller - Killani test :- 5 ml of each extract was treated with 2ml glacial acetic acid containing 1drop of ferric chloride ($FeCl_3$) solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring of interface indicates a deoxysugur characteristic of cardenolides. A violet ring appeared below the brown ring,while in the acetic acid layer, a greenish ring was formed gradually throughout thin layer .

2. Legal's test: - The plant extract was treated with pyridine and alkaline sodium nitroprusside solution was added, blood red colour appeared.

3. Baljet's test: - The plant extract was treated with picric acid or sodium picrate, orange colour is appeared.

Table 1: Phytochemical screening of *T.Cardifolia*

S.No.	Phytochemical constituents	Aqueous extract
	Alkaloids	+
	Flavonoids	+
	Phenol	+
	Saponins	-
	Steroids	+
	Tannins	-
	Terpenoids	-
	Cardiac glycosides	+

+ shows present and – shows absent

Collection of Blood Samples

Fresh blood samples were collected from Pt.J.N.M.College at Raipur. The blood samples were collected in EDTA vials and stored at about 4*c temperature and used within 24 hours of collection.

Sickling Reversal Test

Two drops of sickle cell disease (Hbss) homozygous, blood were mixed with 2 drops of freshly prepared 2% metabisulphite and covered tightly to avoid air from going in .This was incubated for 30 min. Two drops of the aqueous extract of *T.cardifolia* stems were added to the mixture. A drop was placed on a clean slide and covered. This was incubated for another 30 min and then observed at x40 magnification Barbara, 1980; Elekwa *et.al*.2005.

Table 2: The effect of aqueous extract of *T. cardifolia* stems on the reversal of sickled RBCs

% reversal (normal shape of cell) of sickle cell

Treatment	Control (Hbss) untreated Hbss	Invitro induction / treated Hbss	After adding aqueous extract of *T. cardifolia* stems
% sickle cell	40.69%	48.31%	25.80%

RESULTS AND DISCUSSION

Phytochemical Screening

The aqueous extract of *Tinospora cardifolia* stems were subjected to qualitative phytochemical screening for detection of phytoconstituents viz alkaloids, flavanoids, phenols,saponins, steroids,tannins,terpenoids & cardiac glycosides. As shown in Table 1, the results revealed the presence of alkaloids, flavanoids, phenols, steroids & cardiac glycosides.

Sickling Reversal Test

Sickle reversal test of Hbss blood is one of the method of for screening of antisickling property. In this method determination of % sickling is done through RBC counting of Hbss (sickle cell disease homozygous) blood sample. Sickling was induced by taking 2% freshly prepared Sodium metabisulfite solution (invitro

induction) to which Hbss blood was added. Aqueous extract of *Tinospora cardifolia* stems applied on induced sickle Hbss blood sample. The RBCs of sickle blood (Hbss) reversed to normal shape and hence the % of sickling decreased and the % of reversal cell (normal shape of cell) increased.

CONCLUSION

The % of sickling of control Hbss (untreated Hbss) was higher than treated Hbss. The % of sickling decreased when the aqueous extract of *Tinospora cardifolia* stems was added to the blood sample after induction of Hbss blood. Therefore it can be concluded that reversal of RBCs of Hbss blood to normal shape (% of reverse cell increase) is due to the presence of bioactive molecules present in the aqueous extract of *Tinospora cardifolia* stems.

REFERENCES

Aima R.K., 2003. Pictorial guide to plants, Dehradun: Natraj publishers 1st edition.

Barbara A.B., 1980. Hematology, principles and procedures, London, Henry kimpton, pp124-126.

Chopra R.N., Nayar S.L. and Chopra I.C., 1956. Glossary of Indian medicinal plants, New Delhi, CSIR.

Elekwa I., Monanu M.O. and Anosike E.O., 2005. Invitro effects of aqueous extracts of Zanthoxylum macrypha roots on adenosine triphophate from human erythrocytes of different genotypes, Biokemistri, **17**(1):19-25.

Harborne J.B., 1973. Phytochemicals methods, Chapman and Hall ltd., pp 49-188.

Kokate C.K., Purohit A.P. and Gokhale S.B., 2007. Pharmacognosy, Nirali prakashan **38**:607-611.

Sofowara A., 1993: Medicinal plants and traditional medicine in Africa, Spectuum books ltd., Ibadan, Nigeria, pp.191-289.

Vaidya D.B., 1994. Materia of Tibetan medicine. Sri Satguru publications, New Delhi.

Yadav R.N.S. and Agarwala M., 2011. Phytochemical analysis of some medicinal plants, journal of phytology, **3**(2):10-14.