

## STUDY OF IN-VITRO ANTI-SICKLING PROPERTY AND SCREENING OF SECONDARY METABOLITES OF AQUEOUS EXTRACT OF *Terminalia chebula*

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### ABSTRACT

The antisickling property of aqueous extract of fruits of *Terminalia chebula* were evaluated invitro antisickling screening by using sickling reversal test. The aqueous extract of this plant exhibited antisickling property when the aqueous extract used on Hbss blood; from sickle red blood cell reverse to normal biconcave shape. Preliminary phytochemical screening of *T. chebula* extract various secondary metabolites present viz- Alkaloids, flavonoids, phenol, saponins, steroids and cardiac glycosides. This present study investigates the invitro antisickling property and screening of secondary metabolites of aqueous extract of *T. chebula*.

**KEYWORDS:** Antisickling Activity, Aqueous Extract, Secondary Metabolites and *T. chebula*.

Sickle cell disease is an autosomal recessive genetical blood related disease, characterized by red blood cells that assume an abnormal sickle shape and known to be one of the disease affecting the population living mostly in India, Africa, South America and Asia World Health Organisation,2007. In India most affective regions are Chhattisgarh, Maharashtra and Orissa. In Chhattisgarh state sickle cell disease is reported in many tribal communities. Drepanocytosis commonly known as sickle cell disease (SCD) is a genetic disease; which is spread all over the world World Health Organisation,2012.About 50 million people around the world are affected by depanocytosis . Every year approximately 3 lakh childrens are born with blood disorders of which 70% are affected by sickle cell anemia Urade BP,2012. Sickle cell disease is most commonly in the black races. It is also found in other races surrounding the mediterian regions Steinberg NH,2004. Sickle cell disease treat with three methods like –medical therapy, nutritional therapy and natural plant products. Higher plants produce rich amount of secondary metabolites that have therapeutic and pharmaceutical uses Balick MJ & Cox PA, 1996; Shetonde OM,2005. Medicinal plants provide beneficial inputs for sickle cell anaemia because of presence of secondary metabolites.

Anti-sickling agents occur in herbal plants can be used for treatment of sickle cell disease. It has been seen that tribal people in Chhattisgarh usually use herbal plants extract for the treatment of sickle cell patients.*T. chebula* has a wide variety of applications in Indian traditional medicine .These include the relief of sickle cell anaemia and related other complications like – fever, joint pain ,weakness, jaundice, inflammation, vaso-occlusive crises, organ damage and haemolysis etc.

### MATERIALS AND METHODS

**A . Collection of plant material :-** The fresh fruits of *T. chebula* were collected from open field. The fresh fruits were air dried on shadow for two weeks . The dry fruits were grinded into powder by using a grinder machine before being subjected to phytochemical screening.



Fresh fruits & twig of *T. chebula*



Dry fruits with powder

**B. 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 continues till the solvent in siphon tube of an extractor becomes 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<sup>0</sup>c for phytochemical analysis Yadav RNS & Agarwala M ,2011.

**C. Preliminary phytochemical analysis:** The extract was screening for the presence of bioactive compounds by using following standard methods Harbone JB,1973;Sofowora A,1993; Kokate CK *et al* 2007.

**Table 1:Result of qualitative Screening of Phytochemicals**

S. No.	Phytochemicals (Secondary Metabolites)	Name of test	Aqueous extract of <i>T.chebula</i> fruits
1.	Alkaloids	Wagner’s test	+
		Hager’s test	+
		Mayer’s test	-
2.	Flavonoids	Shinoda test	+
		Lead acetate test	+
		Zinc HCL test	+
		Alkaline reagent test	-
3.	Phenol	Ferric chloride test	-
		Lead acetate test	+
4.	Saponins	Foam test	-
5.	Steroids	Salkowski test	+
		Sulfur test	+
6.	Tannins	Braemer’s test	-
		Ferric chloride test	-
		Alkaline reagent test	-
7.	Terpenoids	Salkowski test	-
8.	Cardiac glycosides test	Keller-Killani test	-
		Legal’s test	+
		Baljet’s test	+

( + ) show present and (-) absent

**D. 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.

**E. Sickling reversal test –** Two drops of Hbss 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 during which time sickling was increased . Two drops of the aqueous extract of *T.chebula* fruits were added to the mixture . A drop was placed on haemocytometer and covered . This was incubated for another 30 min and then observed at x40 magnification Barbara,1980; Elekwa *et.al.*2005.(Figure, 1)



**Figure1:Sickle red blood cells**

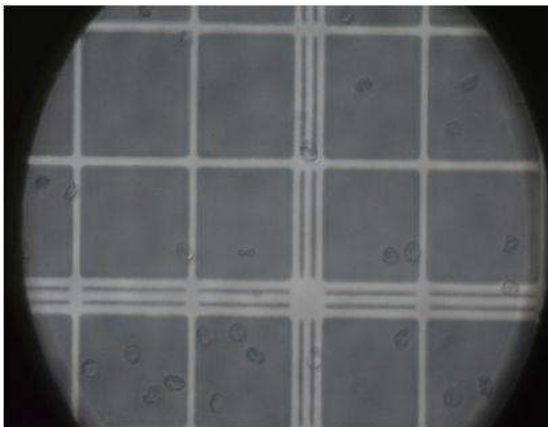
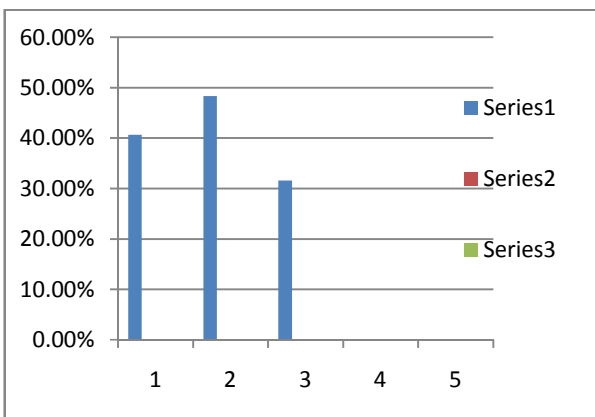


Figure2:Treated sickle red blood cells through aqueous extract of *T. chebula*

Table 2: The reversal of sickling effect of aqueous extract of *T. chebula* ( plant part-fruits) % of sickling

Control (Hbss)	Invitro induction	Aqueous extract of <i>T. chebula</i> fruits
40.69%	48.31%	31.57%



Graph 1: Series 1 – Contrl Hbss, Series 2 – Invitro induction of Hbss, Series 3 – Aueous extract of *T. chebula* ( Fruits) applied on Hbss

**RESULTS AND DISCUSSION**

**Phytochemical screening-** The aqueous extract of *T. chebula* were subjected to qualitative phytochemical screening for detection of secondary metabolites 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 method of invitro antisickling screening. In this method determination of % sickling through Hbss counting . The % sickling of control Hbss is 40.69 % .The % of of induced Hbss (invitro induction )

is 48.31% . Sickling induced by 2% freshly prepared Sodium metabisulfite solution and when the aqueous extract applied on induced Hbss % of sickling is 31.57% . Aqueous extract of *T. chebula* fruits (1 ml) applied on induced sickling Hbss blood , from the sickle blood (Hbss ) reverse to normal biconcave shape. So the % of sickling decreased because of the no. of cells reverse to normal biconcave shape of red blood cells . In aqueous extract of *T. chebula* fruits no. of significant secondary metabolites present so,this secondary metabolites have good anti-sickling and antioxidant properties .Secondary metabolites flavonoids, phenol and cardiac glycosides stabilize and strength of red blood cells and protect red blood cells from harmful free radicals. (Table 1, 2, Figure 2 & Graph 1)

**CONCLUSION**

The % of sickling of untreated Hbss higher than treated Hbss. The % of sickling decreased when the applied of aqueous extract of *T. chebula* fruits on induced Hbss blood that mean Hbss blood reverse to normal biconcave shape .

**REFERENCES**

Balick M.J. and Cox P.A., 1996. Plants, people and culture, The science of ethnobotany, The scientific American library, Newyork.

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

Elekwa I., Monanu M.O. and Anosike E.O., 2005. Invitro effects of aqueous extracts of *Zanthoxylum macrypha* roots on adenosine triphosphate 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 edition, pp 607-611.

Shetonde O.M., 2005. Phytochemical investigation of a Congolese plant *Biophytum umbraculum* (Oxalidaceae) M.Sc. thesis, University of Botswana.

Steinberg N.H., 2004. Sickle Cell Disease, *Hematology*, 35.

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

- Urade B.P., 2012. Incidence of sickle cell anaemia and thalassaemia in Central India, *Open Journal of Blood Diseases*, **2**:71-80.
- World Health Organization, 2007. Management of haemoglobin disorders, Nicosia, Cyprus; 16-18. ( Report of a joint WHO –TIF meeting).
- World Health Organization, 2012. Drepanocytose of autres hemoglobinopathies centre/factsheets/fs308/fr/index.html.
- Yadav R.N.S. and Agarwala M., 2011. Phytochemical analysis of some medicinal plants, *Journal of phytology*, **3**(2):10-14.