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ANTISICKLING PROFILE OF TWO ETHNOMEDICINAL PLANT RECIPES OF EUPHORBIACEAE AND ASTERACEAE FAMILY

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ABSTRACT

Two ethnomedicinal plant recipes of Euphorbiaceae and Asteraceae used in the management of Sickle Cell Anaemia (SCA) were studied for their antisickling activities. Using methanolic extracts, *in vitro* antisickling activities were evaluated using p-hydroxybenzoic acids and normal saline as positive and negative controls, respectively. Phytochemical screening of the extract revealed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannins in recipe 1 except that alkaloids and flavonoids were absent in recipe 2. Antisickling activities of extracts showed 80.1% inhibition by recipe 1 whereas 62.3% in recipe at 120 min incubation. It justifies the use of these plants by traditional practitioner in the treatment of anemia.

KEYWORDS: **Antisickling activities, ethnomedicinal plants, sickle cell anaemia.**

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INTRODUCTION

Sickle cell anemia, is a hereditary disease in which the 'SS' individual possesses an abnormal beta globin gene characterized by abnormal sickle shaped red blood cells (RBCs). In this disease replacement of glutamic acid by valine occurred at position 6, which leads to the devastating clinical manifestations of sickle cell disease[1] . This mutation causes dire reduction in the solubility of sickle cell hemoglobin (HbS) and polymerization of HbS molecule to form long crystalline intracellular mass of fibers which changes shape of erythrocytes into a sickle shape[2] . Sickle cell disease (SCD) affects about five million people yearly in the world. In India there are about 20 million people suffering from this disease[3] . It is one of the most ignored and painful disease of 21st century in third world countries including India. The prevalence of SCD in various castes and communities is 9.4 - 22.2% in endemic areas of Madhya Pradesh, Rajasthan and Chhatisgarh[4] . The disease remains undiagnosed because of large poor, illiterate population and insufficient diagnosis facilities. It is estimated that 4500 newborn babies with sickle cell disease are expected to be added every year and about 18,000 pregnancies are at risk annually. The current available treatment includes blood transfusion, costly medicines, bone marrow transplantation and Hematopoietic Cell Transplantation which increases cost burden Medicinal plants were used in some parts in the treatment of SCD[5,6,7,8] . The bioactive ingredients having therapeutic activity used in traditional practice are mostly unidentified. So there is need to find out phytochemicals that are economical, easily available and having fewer side effects. There is always a need to find out a remedy which is more economical and easily available from these plants. The proposed research work is one step forward to find out a potent product from the plants which can reduce the suffering of people having sickle cell disease. This study was aimed at collecting and identifying plant species constituting two different recipes for evaluation of the plant recipes for *in vitro* antisickling activities, which acclaimed success by traditional healers and also carrying out phytochemical tests of the two recipes.

MATERIALS AND METHODS

Collection of plant materials and preparation for analysis

The medicinal plants *Baliospermum montanum* (Willd) Muell-Arg. (Root), *Bridelia retusa* L. (Stem, Bark, Fruit), *Euphorbia hirta* Linn., *Euphorbia antiquorum* Linn. (Root, Bark, Stem), *Phyllanthus reticulatus* Poir (Bark), *Blumea lacera* (Burm. F.) DC. (Leaves,Roots), *Eclipta ptostrata* Linn.(Leaves, seeds and Roots), *Elephantopus scaber* Linn. (Roots and Leaves), *Cinchorium intybus* Linn. (Leaves), *Wedia chinesis* (Osbeck) Merrill (Whole plant) were the ingredients of the recipe 1 and 2 as given in table 1 are collected by the guidance of traditional herbal healers and the folks who have good knowledge of the use of medicinal plants for the treatment of anemia. The plants were identified as per the description given in Ethno-medicinal diversity used by tribal's of central India[9]. Two recipes used for study consisting of fresh roots, barks and leaves were collected from the central region of India and identified at the Herbarium of the Department of Botany, Govt M.V.M., Bhopal and provided a specimen no.: Bot/08/2015They were air dried and made into powdered. Each recipe was dried thoroughly and blended to powder form. They were stored in dry containers and labeled accordingly. Water soluble fractions of the powdered recipes were obtained to test antisickling activity.

Extraction of the water soluble fractions

50 g of each of the powdered plant materials was put into 500 ml conical flasks and 300 ml of methanol was poured over it with intermittent stirring for 5 days. After that, the extracts were syringe filtered (Whatman, USA) and evaporated to dryness on vacuum concentrator (Eppendorf) and then dispensed into labeled vials and stored in the refrigerator at 4°C for subsequent use.

Collection of blood samples and screening for sickle cell anemia

Five milliliters (5 ml) of blood was collected in EDTA bottles from 186 anemic participants, between 10 – 50 yrs of both gender. A written informed consent was read and signed by all patients participating in the study. The research project has an approval with reference no. PU/CSRD/SI/30. All experiments were performed with fresh blood. In order to confirm their SS nature, the above-mentioned blood samples were first characterized by D-10 HPLC (Bio Rad). Screening was done through sodium metabisulfite test. Other tests viz. complete blood count, blood smear used for screening of sickle subjects.

Antisicking activity evaluation

The modified method was used to carry out antisickling activities of two different methanolic extracts[10] . The packed erythrocytes were collected by centrifugation and vials were washed 3-4 times with 1 ml sterile normal saline per 5 ml of blood. Centrifugation was done for 5 min at 2000 revolution per minutes to remove supernatant. Washed erythrocytes and different extracts were mixed in equal quantity (1 ml) in uncovered test tubes and mixed together. Samples were taken from the different mixtures and the remaining mixtures incubated at 37°C for 3 h with occasional shaking. To deoxygenate the system 2%, 0.3 ml sodium metabisulphite were added, mixed thoroughly and sealed with liquid paraffin. Samples were taken in triplicates from the different mixtures at 0 min to 30 min interval with consecutive five reading with incubation of system at 37°C. Smear of each sample was prepared on microscopic slide, stained with giemsa stain and microscopically examined under oil immersion light microscope. The numbers of both sickled and unsickled red blood cells were counted and thereby percentage of unsickled cells determined. Two types of controls, a positive control, p-hydroxybenzoic acid (5 mg/ml) used to reverse the sickling in HbSS blood cells and normal saline used as negative control were employed in this biological testing. A. The blood sample collected from a particular patient was used for testing of each set of experiment.

Table 1
Recipe 1 from plants with Euphorbiacae family

Botanical name	Part used
Baliospernum montanum (Willd) Muell – Arg.	Root
Bridelia retusa L.	Stem, Bark, Fruit
Euphorbia hirta Linn	Root, stem, leaves
Euphorbia antiquorum Linn.	Root, bark, stem
Phyllanthus reticulatus Poir	Bark

*****Derived from Gangarde et al., 2003.***

Table 2
Recipe 2 from plants with Asteraceae family

Botanical name	Part used
Blumea lacera (Burm. f.) DC	Leaves and root
Eclipta prostrata (Linn.)	Leaves, seed, roots
Elephantopus scaber Linn.	Roots and leaves
Cinchorium intybus Linn.	Leaves
Wedia chinesis (Osbeck) Merrill	Whole plant

*****Derived from Gangarde et al., 2003.***

Phytochemical tests

The two recipes were screened for their phytochemical constituents. Powdered samples were used to test for alkaloids, saponins, tannins, anthraquinones, cardiac glycosides (cardenolides) following established protocols[11, 12].The botanical names, plant parts, used and known chemical constituents of the plants making up Recipes 1 and 2 are shown in Tables 1 and 2.

RESULTS AND DISCUSSION

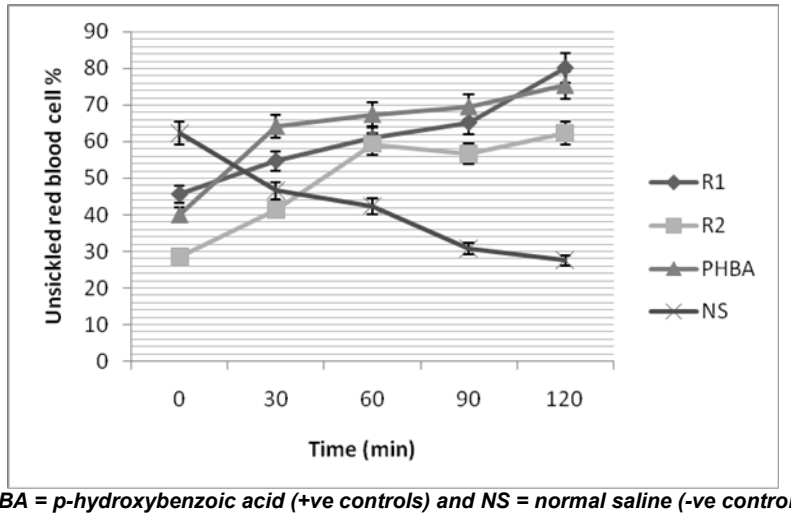
The effect of methanolic extracts of two recipes in inhibiting red blood cell sickling shown in Table 3. Among them Recipe 1 was more active with 80.1% inhibition at 120 min than Recipe 2 which showed 62.3% inhibition at 120 min. As shown in Figures 2, methanolic extracts of Recipe 1 demonstrated more sickling inhibition than Recipe 2. The *p*-hydroxybenzoic acid control showed more sickling inhibition than the methanolic extracts of the Recipe 2 while slightly lower ability to inhibit sickling than Recipe 1. Incubation with control normal saline showed relatively no inhibition and majority of the cells remained sickled after incubation up to 120 min.

Table 3
Antisickling activities of methanolic extracts of Recipes 1 and 2 using p-hydroxybenzoic acid and normal saline as controls.

Time of incubation (min)	% of unsickled red blood cells			
	R1	R2	PHBA	NS
0	45.6	28.6	40	62.2
30	54.7	41.4	64.2	46.6
60	60.9	59.3	67.4	42.3
90	65.2	56.7	69.5	30.7
120	80.1	62.3	75.4	27.5

R1, R2: Recipe 1, 2; PHBA: p-hydroxybenzoic acid; NS: Normal saline
All values are means of triplicate determinations.

Figure 2
Effect of extract of Recipe 1 (R1) and Recipe 2 (R2) on sickled red blood cells.



PHBA = p-hydroxybenzoic acid (+ve controls) and NS = normal saline (-ve controls).

Phytochemical screening showed the presence of anthroquinone, saponins, cardiac glycosides, saponin and tannins in both recipes. However, the presence of alkaloids and flavonoids was occurred only in recipe 1 while it was absent in recipe 2

.

Table 4
Phytochemical screening of Recipe 1 and Recipe 2.

Secondary metabolite	Recipe 1	Recipe 2
Alkaloids	+	-
Anthroquinones	+	+
Cardiac glycosides	+	+
Flavonoids	+	-
Saponin	+	+
Tannins	+	+

The results obtained in the present study show that Recipes 1 and 2 exhibited substantial antisickling activity. The methanolic extracts of these two recipes showed significant inhibitory effect on sodium metabisulphite induced sickling. Recipe 1 exhibited a maximum 65.2% inhibition at 90 min incubation which increased rapidly to 80.1% at 120 min compared 75.4% inhibition demonstrated by *p*-hydroxybenzoic acid at the same time of 180 min. The methaolic extract of Recipe 1 showed a better activity which was sustained even after 180 min of incubation than Recipe 2. Thus recipe 1 could be a better remedy for sicklers than the standard *p*-hydroxybenzoic acid. The efficacy of an antickling agent in vitro must be assessed by a set of reproducible criteria and act effectively and rapidly in severe crises[13]. Similarly, it was identified that *Euphorbia hirta* L. have *in vitro* sickling inhibitory effects and still not used in another recipe for antisickling activity. Anthocyanin extract from *Euphorbia hirta* L. showed 0.659 nm absorbance on 0 min. and it was decreased to 0.450 on 60 min incubation at 450 nm. The absorbance was leas as compared to HbSS sample (0 min: 0.392nm and 60 min: 0401nm)[1] . Presence of anthocyanin may be responsible for more antisickling activity of Recipe 1 compared to Recipe 2.
Phytochemical screening of recipe 1 and 2 for secondary metabolites exhibited presence of alkaloids, anthroquinone, cardiac glycosides, flavonoids, saponin and tannins. Alkaloids and flavonoids were present in recipe 1 but was absent in recipe 2. Alkaloids were used as nerve stimulant, muscle relaxant[14]. Alkaloid may be useful in alleviating symptoms of pains. Anthroquinone increases peristaltic action. Flavonoids possess potential pharmacological activities such as antioxidant activity, vitamin C sparing activity[15]. Antisickling activity of recipe 1 may be due to the presence of antioxidant activity. The presence of cardiac glycosides may be potent in curing cardiac insufficiency, coughs and circulatory problems. Tannins are phenolic derivatives, may be useful in cleansing the surface of the skin ulcers that develop as a result of sickle cell disease[16].Result obtained from experiments showed more than 50% of sickled erythrocytes were reverted at 180 minutes in both recipes. Gradual administration of doses would reduce both frequency and duration of crises. *Euphorbia hirta* L. of recipe 2 have been reported to have antisickling activity[1]. Root extract of *Baliospermum montanum* exhibited immunomodulatory activity by increasing neutrophil function[17]. Fruit extract of *Bridelia retusa* showed anti-inflammatory activity [18] and *Phyllanthus reticulatus* reported hepatoprotective activity hence beneficial for management of SCD. Also, phototherapy has fewer side effects than synthetic entity, were cost effective and importantly they were edible to suppress the symptoms of Sickle cell anemia. Therefore, recipe 1 could be better for management of SCD. Further, isolation and characterization of secondary metabolites from this recipe 1 are recommended.

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