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Evaluation of Hemolytic and Anti-Hemolytic Activity of the Aerial Parts of *Sonchus Oleraceus* Extracts

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ABSTRACT

Plants have been used as alternative remedy for the treatment of various ailments since ancient times. The present study aimed to evaluate some of the biological activities of the plant *Sonchus oleraceus in vitro* including the hemolytic and antihemolytic effect. Hydromethanolic 80% and aqueous extracts of the aerial parts of plant were prepared using ultrasound-assisted extraction. Phyto-chemical analysis, hemolytic and anti-hemolytic activity of both extracts of *Sonchus oleraceus* were assessed. Phyto-chemical screening revealed the presence of tannins, carbohydrates, flavonoids, saponins, and phenolic compounds in both extracts. Hemolytic activity assessment has been conducted, using spectrophotometric method. The extracts had low hemolytic effect towards human erythrocytes in concentration-dependent manner.The extracts showed moderate membrane stabilizing effect against hypotonic-induced hemolysis at concentration range of (250-1000 µg/ml). This study also showed that extracts had potential antioxidant activity through the inhibition of H_2O_2 induced hemolysis. Both extracts showed remarkable antihemolytic activity against H_2O_2 induced hemolysis at all tested concentrations. It was concluded that the extracts of *S. oleraceus* manifest low hemolytic effect, and had antihemolytic activity. However, these effects need to be confirmed using *in vivo* models.

KEYWORDS: *Sonchus Oleraceus*; Hemolytic activity; Erythrocytes membrane; Membrane-stabilizing property; Antihemolytic; Antioxidant.

Introduction

Since the beginning of human civilization, medicinal plants have been used for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have hence been isolated from natural sources (Shwetha et al., 2016). According to the World Health Organization (WHO) estimate, more than 80% of the population of the developing countries rely on conventional plants for initial health care. Only in Asia, medicinal plants has a big impact on economy and primary health care. There are approximately 6,500 species used for curative purpose in Asia (Asif et al., 2014). However, use of herbal drugs in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs (Hossain et al., 2014). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Saxena et al., 2013). Herbal products are often perceived as safe because they are "natural"(Mannan et al., 2015). However, because of the numerous side effects of the synthetic drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications (saxena et al., 2013). Despite this fact inherent toxicity of some of their constituents should not be underestimated, and even nontoxic herbs can have side effects if inappropriately dosed (Colegate and Molyneux, 2008).

It has been reported that many plants used as food or in traditional medicine have mutagenic, cytotoxic and genotoxic effects (Lohith et al., 2013). Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes (Manthey et al., 2001). Plant extracts can positively affect the red cell membrane (Freitas et al., 2008) and many plants have serious adverse effects, which include induction of hemolytic anemia (Zohra and Fawzia, 2014).Therefore, research on medicinal plants is one of the leading areas of research globally (Shailesh et al., 2011), and it is of utmost importance that efficacy and toxicity risks or potential hemolytic activity of medicinal plants are evaluated (Zohra and Fawzia, 2014). Hemolysis assay is an extremely sensitive method for cytotoxic studies with wide range of phytochemicals effects (Lohith et al., 2013). Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer, by which hemoglobin and other internal cell components are

released into the surrounding fluids (Muthu and Durairaj, 2015; Zohra and Fawzia, 2014). Erythrocytes have been used as a model system by a number of workers for the study of interaction of drugs with membranes (Sumathi and Anuradha, 2016), because it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood; moreover, its membrane has similarities with other cell membrane (Muthu and Durairaj, 2015; Zohra and Fawzia, 2014).

The plant *sonchus oleraceus* belongs to Asteraceae family, which is the largest and the most cosmopolitan of the flowering plants and is probably the most widespread in the Mediterranean (Attard and Cuschieri, 2009). It is an erect annual herb (Singh and Jain, 2014), has known worldwide for its folk medicinal properties including uses as antimalarial, for headaches, general pain-relief. Antioxidant (Albalawi et al., 2015) anticancer, digestive, purgative, emollient, blood purifier (Singh and Jain, 2014), anti-inflammatory, antidiarrheal, diuretic, toothache remedy, sedative, heart medicine, stop bleeding, narcotic, insecticide (Jimoh et al., 2011). In this context, this study was aimed to screen the plant for its phytochemical constituents in aqueous and methanolic extracts, evaluate the hemolytic, and the anti-hemolytic activities of both extracts *in vitro* by using hypotonic solution and H_2O_2 induced hemolysis.

Materials and Methods

Chemicals and Equipment

Chemicals

From Merck (Germany): Ferric Chloride, Methanol, Sulphuric Acid, NaCl, KCl, Na_2HPO4, KH_2PO4, Ascorbic acid.

BDH (England): Hydrochloric Acid, α Naphthol, Magnesium Turnings, Lead Acetate.

Equipments: Ultrasonic Bath (Hwashin Power Sonic 405), Centrifuge (HeraeusMegafuge 2.0R), Rotary evaporator (HeidolphLaborata 4000, Germany), spectrophotometer (Jasco, V-650, Japan), PH meter (Crison, Spain).

Plant material collection: Aerial parts of *S. oleraceus* plant were collected at flowering stage. The plant material were authenticated by an expert at the Faculty of Agriculture -Aleppo University, Syria.

It was cleaned and air dried at laboratory temperature. Then, it was powdered using mechanical grinder and kept in airtight glass container until use.

Preparation of extracts *(Pandey and Tripathi, 2014):* 50 gr of plant powder was extracted using 250 ml of each solvent [aqueous methanol solvent (methanol (80): water (20)), water] in ultrasonic waves apparatus for 25 minutes with a frequency of 40 khz at 30 ºC. Extractswere clarified by filtration and the residual was re-extracted three times by the same procedure.After that, extractswere evaporated under reduced pressure in a rotary evaporator attached to a vacuum pump at 40 ºC until dryness, then they put into petri dishes in a desiccator containing dry silica.

Extraction yield was calculated depending upon this formula:

yield = (dry extract weight/ plant powder weight) × 100

Finally, the concentrations of both extracts were prepared using phosphate buffer saline (PBS). The dried extracts obtained were used for the following experiments.

Qualitative Analysis on Phytochemical Constituents of *S. Oleraceus* Plant

Detection of carbohydrates *(Ahmad et al., 2012): Molisch test:* The extract (5 mg) was dissolved in 5 mL of distilled water and few drops of Molischre agent (1% methanolic alpha-naphthol) was added, shaked well and concentrated H_2SO_4 was added from sides of the test tube, violet ring formation at the junction of two liquids indicates the presence of carbohydrates.

Detection of saponins *(Anbarasi and Vidhya, 2015): Foam Test:* Distilled water (5 mL) was added to each plant extracts (5 mg) and shaked in a graduated cylinder for 15 min length wise. Formation of 1cm foam indicates the presence of saponins.

Detection of phenolic compounds

(Anbarasi and Vidhya, 2015):

Lead acetate test: The extract (5 mg) was dissolved in 5 mL of distilled water and 3 mL of 10% lead acetate solution was added. A bulky white precipitates indicates the presence of phenols.

Detection of tannins *(Anbarasi and Vidhya, 2015):*

Ferric chloride test: The extract (5 mg) was dissolved in 5 mL of distilled water and few drops of neutral 5% ferric chloride solution were added. The appearance of green color indicates the presence of tannins.

Detection of flavonoids *(Rajagopal et al., 2016):*

Shinoda test: Few magnesiun turnings and few drops of concentrated HCl from the sides of the test tube were added to 5 mL of aqueous solution of the dry extract (5 mg). The appearance of pink, crimson red color indicates the presence of flavonoids.

Hemolytic Activity Assessment (*in vitro* study)

Preparation of human Red Blood Cells (HRBC) Suspension *(Ukwuani and Ahmad, 2015; Lakshmi et al., 2014):* About 5 mL of blood from healthy volunteers was collected in a tube containing heparin. The blood was centrifuged at 1500 rpm for 3 min. The supernatant was collected and plasma was discarded. The pellet was washed for 3 times using PBS solution and centrifuged at 1500 rpm for 5min. The cells were resuspended in PBS to 10%.

Hemolytic Activity *(Lakshmi et al., 2014)*

To 1 mL of cell suspension, 1 mL of different concentration of plant extract (10, 50, 100 and 250, 500, 1000 μg/mL) in phosphate buffer saline (pH 7.2) was added. The mixture was incubated at 37 ºC for 30 min and centrifuged at 2500 rpm for 10 min. The free hemoglobin in the supernatant was measured using spectrophotometer at 540 nm. The phosphate buffer

saline and distilled water was used as negative and positive hemolytic control.

% hemolytic activity was calculated using the given formula:

$$\text{Hemolysis \%} = \frac{(A_t - A_n)}{(A_c - A_n)} \times 100 \quad \text{.....(1)}$$

A_t- Absorbance of test sample.

A_n- Absorbance of control (saline).

A_c- Absorbance of control (water).

Anti-Hemolytic Activity Assessment

The anti-hemolytic activity assessment was performed using two different methods.

Hypotonic Solution Induced Hemolysis (Anti-Inflammatory Activity) *(Ukwuani and Ahmad, 2015; Patel and Desai, 2016; Ukwuani and Hassan, 2015):* The reaction mixture consists of 1.0 mL of test sample of different concentration and 0.5 ml of 10% HRBC suspension, 0.5 mL hypo saline (NaCl 0.03%). Control was prepared without extracts. The mixture was incubated at 37 ºC for 30 min and centrifuged at 2500 rpm for 10 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 540 nm. The percentage of hemolysis and membrane stabilization or protection was calculated by using the following formula;

Hemolysis % =

(Absorbance of test sample / Absorbance of control) × 100 .....(2)

Protection % = 100 – [hemolysis] .....(3)

H_2O_2 Induced Hemolysis *(Joshan and Rawal, 2012):* 1 mL of extract of different concentration was added to 0.5 mL of HRBC suspension (10%). The mixture was incubated for 5 minat room temperature and then 0.5 mL of 0.3% H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. A control was prepared without extracts.

The mixture was incubated at 37 ºC for 240 min and then centrifuged at 2500rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation. The percentage of hemolysis and membrane stabilization or protection was calculated by using the above formula (1 + 2).

Statistical analysis: Data obtained were analyzed using Statistical Package for Social Science (SPSS version 17.0). Values are expressed as mean ± SD for ten replicates. The statistical significance differences were analyzed using Independent samples t-test, and values of $p < 0.05$ were considered statistically significant.

Results

Yields of Plant Extracts

The yields of the dried extracts were as follows: aqueous extract 7.5% and methanolic extract 8.6% (Table 1).

TABLE 1

The yields percentages of *S. oleraceus* extracts.

Extraction Method	Solvent Used	
	Methanol 80%	water
ultrasound-assisted extraction	% 8.6	7.5 %

Phytochemical Analysis

S. oleraceus extracts were subjected to Preliminary phytochemical analysis to check the presence of phytochemical compounds. The phytochemical screening showed the presence of bioactive phytocompounds such as carbohydrates, Saponins, phenolic compounds, Flavonoids and tannins in both extracts (Table 2).

TABLE 2

Phytochemical screening results of *S. oleraceus* extraxts.

Constituent	Plant extract	
	Methanolic extract	Aqueous extract
Tannins	+	+
Carbohydrate	+	+
Flavanoids	+	+
Phenolic compounds	+	+
Saponins	+	+

Hemolytic Activity

Hemolytic activity of the aerial parts of *S. oleraceus* methanolic and aqueous extracts were screened against normal human erythrocytes. Extracts exhibited low hemolytic effect towards human erythrocytes in comparison of positive control (distilled water). Results indicated that extracts didnot show harmful effect towards human erythrocytes, they possess minimum hemolytic activity (at concentration 10 µg/mL) andmaximum hemolytic activity (at concentration 1000 µg/mL). Lysis of erythrocytes was found to be increased with an increase of extract concentration (Table 3 and figure 1). There was significant difference ($p<0.05$) between methanolic and aqueous extracts.

TABLE 3

The percentages of hemolytic activity by *S. oleraceus* extracts.

Concentration (µg/ml)	Methanolic extract	Aqueous extract
	Hemolysis %	Hemolysis %
0.6 ± 0.33	0.9 ± 0.57	**10**
1.4 ± 0.36	1.9 ± 0.55	**50**
2.1 ± 0.53	2.9 ± 0.71	**100**
3.3 ± 0.6	4.2 ± 0.63	**250**
4.1 ± 0.63	5.2 ± 0.56	**500**
5.8 ± 0.97	6.7 ± 0.67	**1000**

All values are represented as mean ±SD

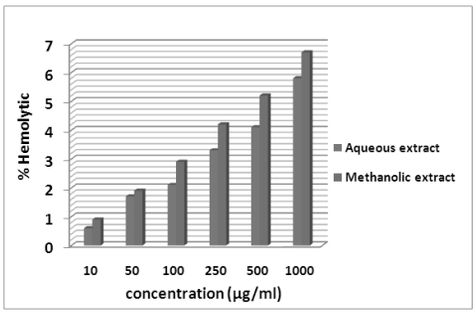

Fig. 1. Hemolytic effect of *S. oleraceus* extracts.

Anti-hemolytic Activity using Hypotonic Solution Induced Hemolysis Assessment

In vitro anti-hemolytic activity of the extracts of *S. oleraceus* was screened against HRBC membrane. Results indicated that the extracts did not show adequate membrane stabilizing effect at concentration range of (10-100 µg/mL) against lysis induced by hypotonic solution, whereas at concentration range of (250-1000 µg/mL), the extracts produced moderate protection, in comparison of sodium diclofenac as positive control (data not shown), with maximum activity of 69.4% and 67.6% (at concentration 1000 µg/mL) in the methanolic and aqueous extract respectively (Table 4 and figure 2). The difference between methanolic and aqueous extracts activities was not significant ($p>0.05$).

TABLE 4

The percentages of protection by extracts using hypotonic solution NaCl (0.03%) induced hemolysis.

Concentration (µg/ml)	Protection %	
	Methanolic Extract	Aqueous Extract
10	48.1 ± 1.25	46.8 ± 3.09
50	49.4 ± 2.18	48.6 ± 2.2
100	55.8 ± 3.72	55.2 ± 2.91
250	64.3 ± 2.41	63.8 ± 2.19
500	67.2 ± 1.79	65.8 ± 1.18
1000	69.4 ± 1.77	68.1 ± 2.39

Protection values are represented as mean ± SD

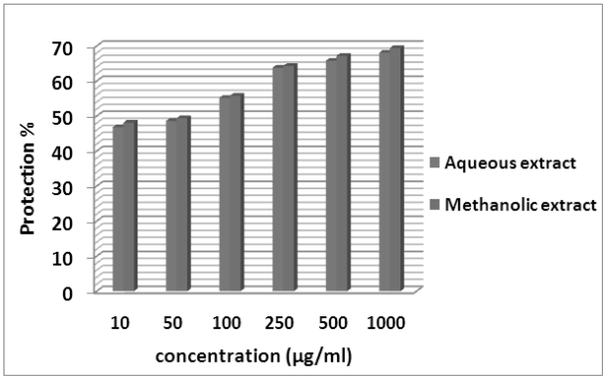

Fig. 2. Antihemolytic activity of *S. oleraceus* extracts using hypotonic solution induced hemolysis.

Anti-hemolytic Activity of H_2O_2 Induced Hemolysis Assessment

The protective effect of *S. oleraceus* extracts against H_2O_2 induced hemolysis was investigated. In general, both extracts showed antihemolytic effect in concentration dependent manner. They exhibited relevant antihemolytic effect at concentration range of (10-1000 µg/mL), in comparison of ascorbic acid as positive control (data not shown), in terms of percentage inhibiting activity which was ranging from 76.7% to 85.6% for methanolic extract and from 75.8% to 83.8% for aqueous extract (Table 5 and figure 3). Statistical study showed that the difference between methanolic and aqueous extracts activities was not significant ($p>0.05$).

TABLE 5

The percentages of protection by extracts using H_2O_2 induced hemolysis.

Concentration (µg/ml)	Protection%	
	Methanolic Extract	Aqueous Extract
10	76.7 ± 2.95	75.8 ± 1.67
50	77.2 ± 2.45	76.2 ± 2.43
100	78.9 ± 3.47	77.8 ± 2.94
250	80.6 ± 2.22	79.3 ± 2.14
500	82.1 ± 1.7	80.7 ± 2.23
1000	85.6 ± 1.89	83.8 ± 2.07

Protection values are represented as mean ±SD

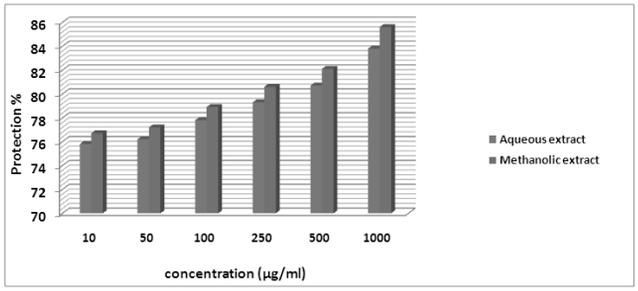

Fig. 3. Antihemolytic activity of *S. oleraceus* extracts using H_2O_2 Induced hemolysis.

Discussion

Yields of Plant Extracts

Ultrasonic waves helps to disruption of biological membranes to facilitate the release of extractable compounds and enhance penetration of solvent into cellular materials (Dai and Mumper, 2010). The ultrasonic extraction method is easily carried out technique and was found to have improvement in the percentage yields and shorter extraction time in comparison with conventional techniques (Žlabur et al., 2015).

It was found that hydromethanolic extract gave higher yield in comparison of aqueous one, because methanol is organic, high polarity solvent andso has ability to extract large range of compounds (Yuliana et al., 2014). Our findings are in agreement with previous investigation by (Sultana et al., 2009) who reported that the higher extract yields were found in the 80% aqueous methanol of selected medicinal plants, included *M. oleifera, Azadirachtaindica, Terminalia arjuna, Acacia nilotica, Eugenia jambolana*and *Aloe barbadenis*.

Phytochemical Analysis

Medicinal plants contain various phytochemical compouds that attribute to their medicinal propertities (Lakshmi et al., 2014). They are playing vital role for the treatment of different types of diseases (Shah et al., 2011).The phytochemical screening showed the presence of carbohydrates, saponins, phenolic compounds, flavonoids and tannins in both extracts.

Hemolytic Activity

Hemolysis is the breakage of the red blood cells (RBC's) membrane, causing the release of the

hemoglobin and other internal components into the surrounding fluid. The evaluation of *in vitro* hemolytic action has been used as screening methodology for various toxic agents. Phytochemicals present in extract could have hemolytic activity (Lakshmi et al., 2014). Therefore, *in vitro* hemolysis tests have been employed by several authors for the toxicological evaluation of different plants (Shobana and Vidhya, 2016).

The present results showed that extracts exhibited low hemolytic effect toward human erythrocytes. This hemolytic activity of extracts is related to their chemical composition. Preliminary phytochemical screening revealed that *S. oleraceus* aerial parts extracts contain saponins. These compounds are presumably the source of the hemolytic activity of extracts prepared from this plant. Saponins are known to have undesirable haemolytic effect (Urbańska et al., 2009). Amphiphilic nature of saponins (i.e. presence of a lipid-soluble sapogenin moiety linked to water soluble sugar chains) enable saponins as surface active compounds with foaming feature to interact with cell membrane. Due to surface activity of saponins these compound have been able to disrupt cell membranes (i.e. form complexes with cell membrane cholesterol) (Amini et al., 2014) causing rupture and release of characteristic hemoglobin pigments (Oliveira et al., 2009), which this lytic action on erythrocytes membrane utilize as saponin detection (Amini et al., 2014). Further more, the hemolytic activities of saponins are related to their chemical composition. Saponins with steroid aglycon have shown more hemolytic activity than those with triterpenoid aglycon (Noudeh et al., 2010).

Anti-hemolytic Activity using Hypotonic Solution induced hemolysis:

Exposure of red blood cells (RBCs) to injurious substances such as hypotonic medium, heat etc., results in the lysis of the membranes (Debnath et al., 2013).

The anti-hemolytic studies reveal the ability of any substance to stabilize the membrane and reduce the extent of lysis of red blood cells (Vidya et al., 2015). It is relevant from the present study that extracts show moderate membrane stabilizing effect at concentration range of (250-1000 µg/mL) (Table 4) and figure (2). The exact mechanism of the membrane stabilization by the extract is not known yet (Sangeetha and Vidhya, 2016; Sumathi and Anuradha, 2016; Rao, 2015). However, a number of investigators have shown that, tannins and flavonoids hadstabilizing action for red blood cell membrane exposed to hypotonic induced lyses (Awe et al., 2009; Oluboade et al., 2015).

The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Kuddus et al., 2012). Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Shirwaikar et al., 2011; Rajalakshmi and Harindran, 2013). So, The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the *in vitro* anti-inflammatory activity (Ananthi and Chitra, 2013).

Anti-hemolytic Activity of H_2O_2 Induced Hemolysis

The oxidation of erythrocytes serves as good model for the oxidative damage of biological membranes (Saradha et al., 2013; Chakraborty and Shah, 2011). Erythrocytes are considered as major target for the free radicals owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the oxygen transport that associated with redox active hemoglobin molecules, which are potent promoters of acid oxygen species (Shobana and Vidhya, 2016; Borra et al., 2013).

The assay principle of this experiment is that H_2O_2 generates peroxyl radicals (ROO-) that attack the erythrocytes to induce the chain oxidation of lipids and proteins, disturbing the membrane organization and eventually leading to hemolysis (Joshan and Rawal, 2012). When red blood cells were treated with extracts along with H_2O_2, marked reduction in hemolysis was found. Both extracts protected the erythrocyte membrane from hemolysis induced by H_2O_2 in concentration-dependent manner. This may be due to the radical scavenging activity of the bioactive compounds present in the extracts. Preliminary phytochemical screening revealed that *S. oleraceus* extracts contain phenolic compounds, which are strongly correlated with Antihemolytic activity (Khalili et al., 2014). Since phenolic compounds appear to function as good electron and hydrogen atom donors, and therefore, be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products (Ebrahimzadeh et al., 2010). Our findings are in agreement with studies showing that phenolic compounds protect RBCs from oxidative stress or increase their resistance to oxidative damage.

Conclusions

S. oleraceus is an important medicinal plant with several medicinal uses in folk and traditional medicine. We are reporting first time the hemolytic and antihemolytic activity of *S. oleraceus* aerial parts extract. The results of current study conclude that the extracts manifest low hemolytic effect and there was significant difference between them, but a great attention should be taken with high concentrations. The study also indicates that the extracts show moderate membrane stabilizing effect against hypotonic solution at high concentrations, that may be due to the presence of tannins and flavonoids but the mechanism is still unclear. The extracts have remarkable antihemolytic activity against H_2O_2 induced hemolysis of the human erythrocytes that may be due to its high levels of phenolic compounds. The difference between methanolic and aqueous extracts antihemolytic activities was not significant. Further investigation is recommended to evaluate the hemolytic

and antihemolytic activity of *S. oleraceus* extract *in vivo* on animals, and to identify and isolate thechemical constituents responsible for the antihemolytic activity in order to determinethe exact mechanism of anti-hemolytic effect.

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References

Ahmad WS, Shah K.W, and Ashfaq MA (2012). Preliminary phytochemical investigation and thin layer chromatography of *rheum emodi*. *Int Res J Pharm,* **3**: 176-177.

Albalawi MAD, Bashir NAO, and Tawfik A (2015). Anticancer and antifolate activities of extracts of six Saudi Arabian wild plants used in folk medicine. *J Life Sci* **9**: 334-340.

Amini E, Nabiuni M, Baharara J, Parivar K, and Asili J (2014). Hemolytic and Cytotoxic Effects of SaponinLike Compounds Isolated From Persian Gulf Brittle Star (*Ophiocomaerinaceus*), *J Coastal Life Med*, **2**: 762-768.

Ananthi T, and Chitra M, (2013). screening of *in vitro* anti-inflammatory activity of *Michelia Champaca LINN. FLOWERS*. *Int J Pharm sci res*, **4**: 721-723.

Anbarasi A, and Vidhya R (2015). Evaluation of in vitro anti-inflammatory activity of *tephrosiapurpurea* (seed). *Asian J. Pharm. Res,* **5**: 83-89.

Asif F, Boby AZ, Alam N, Taraquzzaman M, Chowdhury SR, and Rashid MA (2014). Canvassing of thrombolytic, cytotoxic, and erythrocyte membrane-stabilizing attributes in *in vitro* screening of Gynocardiaodorata. *J Anal Sci Technol,* **5**: 2-7.

Attard E, and Cuschieri A (2009). *In vitro* immunomodulatory activity of various extracts of Maltese plants from the Asteraceae family. *J Med Plants Res,* **3**: 457-461.

Awe EO, Makinde JM, Adeloye OA, and Banjoko SO (2009). Membrane Stabilizing Activity of *Russelia Equisetiformis*, Schlecht & Chan. *J Nat Prod*, **2**: 3-9.

Borra SK, Gurumurthy P, Mahendra J, Jayamathi KM, Cherian CN, and Chand R (2013). Antioxidant and Free Radical Scavenging Activity of Curcumin Determined By Using Different *in vitro* and *ex vivo* Models. *J Med Plants Res*, **7**: 2680-2690.

Chakraborty D, and Shah B (2011). Antimicrobial, Antioxidative and Antihemolytic Activity OF *Piper Betel* Leaf Extracts. *Int J Pharm Pharm Sci*, **3**:192-199.

Colegate SM and Molyneux RJ (2008). Bioactive Natural Products: Detection, Isolation, and Structural Determination, 2nd ed. CRC Press Taylor & Francis Group, New York.

Dai J and Mumper RJ (2010). Plant Phenolics: Extraction, Analysis and their Antioxidant and Anticancer Properties. Mol, **15**: 7313-7352.

Debnath PC, Das A, Islam A, Islam A, and Hassan M (2013). Membrane Stabilization e A Possible Mechanism of Action For The Anti-Inflammatory Activity of a Bangladeshi Medicinal Plant: *Erioglossumrubiginosum* (Bara Harina). *Pharmacogn J*, **5**: 104-107.

Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B, and Rahmani ZP (2010). Antioxidant and Antihaemolytic Activities of the Leaves of *Kefe cumin* (Laser trilobum L) Umbelliferae. *Trop J Pharm Res*, **9**: 441-449.

Freitas MVD, Netto RC, Huss JCD, Souza TM, Costa JO, Firmino CB, and Silva NP (2008). Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. *Toxicol In Vitro*, **22**: 219-224.

Hossain MM, Ahamed SK, Dewan SMR, Hassan MM, Istiaq A, Islam MS, and Moghal MMR (2014). *In vivo* antipyretic, antiemetic, in vitro membrane stabilization, antimicrobial, and cytotoxic activities of different extracts from Spilanthe-spaniculata leaves. *Biol Res,* **47**: 2-9.

Jimoh FO, Adedapo AA, and Afolayan AJ (2011). Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchusoleraceus. Rec. Nat. Prod*, 5: 29-42.

Joshan D.S, and Rawal S (2012). Comparative evaluation of antioxidant and antihemolytic capacities of plants of indian origin using multiple antioxidant assays. *Int J Phytopharm*, **2**: 107-115.

Khalili M, Ebrahimzadeh MA, and Safdari Y (2014). Antihemolytic Activity of Thirty Herbal Extracts in Mouse Red Blood Cells. *ArhHig Rada Toksikol*, **65**: 399-406.

Kuddus MR, Alam MS, Chowdhury SR, Rumi F, Sikder MD, and Rashid MA (2012). Evaluation of Membrane Stabilizing Activity, Total Phenolic Content, Brine Shrimp Lethality Bioassay, Thrombolytic and Antimicrobial Activities of *Tagetespatula*L. *J PharmacognPhytochem*, **1**: 57-62.

Lakshmi G, Smitha N, Ammu SV, and Priya L, and Bhaskarakv (2014). Phytochemical profile, *in vitro* antioxidant and hemolytic activities of various leaf extract of *Nymphaea nouchali*linn: an *in vitro* study. *Int J Pharm Pharm Sci*, **6**: 548-552.

Lohith K, Vijay R, Pushpalatha KC, and Joshi CG (2013). *In-vitro* cytotoxic study of moullava*spicata* (dalz.) nicolson leaf extract. *Int Res J Pharm. App Sci* **3**: 38-42.

Mannan MM, Akhter R, Shahriar M, and Bhuiyan MA (2015). Evaluation of *in vitro* membrane stabilizing activity and thrombolytic activity of *averrhoabilimbi*leaf extracts. *Int J Pharm,* **5**: 357-360.

Manthey JA, Grohmann K, and Guthrie N (2001). Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem*, **8:** 135-53.

Muthu S, and Durairaj B (2015). Inhibitory effect of hydroethanolic extracts Of *Annona Muricata*on human platelet aggregation and hemolysis *in vitro*. *Int J Pharm Pharm Res (IJPPR,)* **2**: 207-214.

Noudeh GD, Sharififar F, Khatib M, Behravan E, and Afzadi M.A (2010). Study of Aqueous Extract of Three Medicinal Plants on Cell Membrane–Permeabilizing and Their Surface Properties. *Afr J Biotechnol*, **9**: 110-116.

OliveiraV, Carneiro A, Cauper G, and Pohlit AMP (2009). *In Vitro* Screening of Amazonian Plants For Hemolytic Activity and Inhibition of Platelet Aggregation in Human Blood. *Acta Amazonica*, **39**: 973-980.

Oluboade OO, Morakinyo MA, Monijesu IJ, Olasunmbo AE, Feyisayo AK, and Ayinke AB (2015). Biological Activities (Anti-Inflammatory and Anti-oxidant) of Fractions and Methanolic Extract of *PhilonotisHastata*(DubyWijk&Margadant). *Afr J Tradit Complement Altern Med*, **12**: 50-55.

Pandey A, and Tripathi S (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*, **2**: 115-119.

Patel D, and Desai S (2016). Phytochemical screening*, in vitro* anti-microbial and anti-inflammatory activity of methanolic extract of *Aster lanceolatuswilld* leaves. *Int J Med Res*, **1**: 26-30.

Rajagopal PL, Premaletha K, and Sreejith KR (2016). Anti-inflammatory screening on the flowers of *trichodesmaindicumlinn*. *World Wide J Multidiscip Res Dev,* **2**: 19-21.

Rajalakshmi GR, and Harindran J (2013). Anti-Inflammatory Activity of *Samaderaindica*Leaves by Membrane Stabilization. *Int J Pharm sci res*, **4**: 721-723.

Rao CS (2015). Study of Pharmacological Anti-inflammatory Activity of *Psidiumguajava*Linn. Leaves and Bark by Tannin Fractions. *J Pharmacogn Phytochem*, **3**: 37-45.

Sangeetha G, and Vidhya R, (2016). *In Vitro* Anti-Inflammatory Activity of Different Parts of *Pedalium murex* (L.). *Int J Herbal Med*, **4**: 31-36.

Saradha M, Paulsamy S, and Vinitha R (2013). Antioxidant and Antihemolytic Activity of an Endangered Plant Species, *hildegardiapopulifolia*(roxb.) schott & endl. *Asian J Pharm clin res*, **6**: 135-137.

Saxena M, Saxena J, Nema R, Singh D, and Gupta A (2013). Phytochemistry of medicinal plants. *J Pharm Phytochem,* **1**: 168-183.

Shah S, Khan K, Shah S, Chishti K, Pirzada S, Khan M, and Farid A (2011). Evaluation of Phytochemicals and Antimicrobial Activity of White and Blue Capitulum and Whole Plant of *Silybum Marianum*. *World App Sci J,* **12**: 1139-1144.

ShaileshG, SeemaK, and DwivediS (2011). *In-Vitro* anti-inflammatory activity of Sarcostemmaacidum Wight. &Arn. Indian herb by Human red blood cell membrane stabilization method. *Int J Pharm Teaching & Practices,* **2**: 184-188.

Shirwaikar A, Devi S and Siju EN (2011). Anti-Inflammatory Activity of *Thespesiapopulnea* fruits by Membrane Stabilization. *Int J Pharm Tech Res*, **3**: 2060-2063.

Shobana S, and Vidhya R (2016). Evaluation of *In vitro* Hemolytic Activity of Different Parts of *Abutilon Indicum* (Linn.). *World J Pharm Pharm Sci*, **5**: 1182-1196.

Shwetha RJ, Tahareen S, and Myrene RD (2016). Antioxidant and anti-inflammatory activity of *tinosporacordifolia*using *in vitro* models. *J Chem Biol Phys Sci*, **6**: 497-512.

Singh GK, and Jain SK (2014). Preliminary phytochemical sereening and in vitro antioxidant activity of extracts of whole plant of Sonchusoleraceusasteraceae. *Res J Pharm Sci* **3**: 1-12.

Sultana B, Anwar F, and Ashraf M (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Mol,* **14**: 2167-2180.

Sumathi S, and Anuradha R (2016). *In vitro* anti-inflammatory acitivty of flower extract of *couroupitaguianensis*aubl. *Innovare J Ayurvedic Sci*, **4**: 16-18.

Ukwuani AN, and Ahmad HB (2015). *In vitro* anti-inflammatory activity of *parkiabiglobosa* fruit bark extract. *Int J Life-SciSci Res (IJLSSR)*, **1**: 8-11.

UkwuaniAN, and Hassan IB (2015). *In vitro* anti-inflammatory activity of hydromethanolic seed, fruit and leave extracts of *capsicum chinense*(red pepper). *Eur J Biomed Pharm sci*, **2**: 57-65.

Urbańska N, Nartowska J, Skorupska A, Ruszkowski D, Giebultowicz J, and Olszowska O (2009). Determination and Haemolytic Activity of Saponins in Hairy Root Culture of *Platycodongrandiforum* A.DC.*herbapolonica*, **55**: 103-108.

Vidya V, Somayaji TY, Pooja S, PATIL S, Fernandes R, and Krishna AB (2015). Assessment of Membrane Stabilization, Antioxidant and Thrombolytic Potential of Lutein- an *in-vitro* Study. *Int J Pharm Sci Res*, **6**: 4478-4483.

Yuliana P, Laconi EB, Wina E, and Jayanegara A (2014). extraction of tannins and saponins from plant sources and their effects on in vitro methanogenesis and rumen fermentation. *J Indonesian Trop Anim Agric,* **39**: 91-97.

Žlabur JS, Voća S, Dobričević N, Brnčić M, Dujmić F, and Brnčić SR (2015). Optimization of ultrasound assisted extraction of functional ingredients from *Stevia rebaudiana Bertoni* leaves. *Int. Agrophys*, 29: 23-237.

Zohra M, and Fawzia A (2014). Hemolytic activity of different herbal extracts used in algeria. *Int J Pharm Sci Res (IJPSR)*, **5**: 495-500.

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