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Short Communication

Hemolytic Activity of *Acacia nilotica* & *Achillea millefolium* on Human Red Blood Cells (*in vitro* Study)

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Abstract
In this study, five concentrations of methanolic extraction of *Acacia nilotica* (10, 20, 50, 100, 250 mg) and six concentrations of *Achillea millefolium* (25, 50, 75, 100, 150, 200 mg) were used to study the extent of their effect on the damage of Red blood cells walls, the study were tested for replicates of blood samples for each concentration matched by controlled blood samples which were not used with the alcohol extract. The absorbance was obtained using a spectral device of a wavelength of 540 nm. The results indicated that there was a marked increase in red blood cell decomposition with the increasing of the concentration for both plants extractions.

Key Words: *Acacia nilotica*, *Achillea millefolium*, hemolytic activity.

الخلاصة
تم في هذا البحث استخدام خمس تراكيز من المستخلص الكحولي المثيلي لأوراق وثمار كلا من نبات الاكاسيا *Acacia Nilotica* (10,20,50,100,250) mg وست تراكيز من المستخلص الكحولي لنبات الاخيليا *Achillea Millefolium* (25,50,75,100,150,200) mg لدراسة مدى تأثيرها على تحلل جدار كريات الدم الحمراء مختبريا لـ ٣٥ مكرر من العينات الدموية لكل تركيز يقابلها عينات من الدم لم يستخدم معها المستخلص الكحولي للنباتين أعلاه وتمت قراءة الامتصاصية باستخدام جهاز المطياف طول موجي ٥٤٠ nm. أوضحت النتائج انه كانت هناك زيادة واضحة في عملية تحلل كريات الدم الحمراء كلما زاد تركيز المستخلص ولكلا النباتين أعلاه.

Introduction

Since the very old days, medical plants were used to treat many diseases , these medical Plants considered as the sources for the outmoded remedy schemes all over the world for a thousands of ages & continue to afford manhood with firsthand therapies.

The exact shrubberies to be used & the approaches of germaneness for these particular conditions did approve through bygone history. Ultimately information concerning medicinal plants were documented in herbals, during the olden times; uses of plants as medicines has concerned the seclusion of active composites, at the inauguration; the seclusion of morphine from opium in the early 19th. epoch [1].

Voluminous plants grounded medicines nowadays work as the basis of new drug sighting, These medicines at the start; took the form of crude medicines for example teas; tincture; powder; poultices & other herbal provisions [2, 3].

Then, Drug finding from remedial plants steered to the separation of early remedies for instance: codeines; cocaines, quinines & digitoxins, in additions to morphine, of which various are still in use [4, 5].

The segregation & description of pharmaceutically dynamic composites gotten from remedial plants still carry on nowadays. Recently; drug discovery procedures have been applied to the site of herbal medicines.

The active principles of many plant kinds are sequestered for straight use as remedies, prime compounds or pharmaceutical representatives [6].

Therefore, Progressive scientific techniques; brought a revision in herbal medicine manufacturing and focusing concentrate on the (Active Principles) (bio-active molecules). yet, lots of treating is essential to develop drugs from their natural sources; taking into consideration that poisonousness of the active molecule is a main issue during drug planning

& haemolytic activity symbolizes a useful starting point for this concern; it offers the primary information for the interfaces stuck between fragments and biological contents at a cellular level. Thus; hemolytic bustle of any composites is an indicator for general cytotoxicity on the way to the normal and vigorous cells [7].

Generally; saponins which present in the plants (a group of phytochemical) bared haemolytic bustle by producing vicissitudes in the red cells membranes. Yet; *In vitro* haemolytic analyze by spectrophotometric techniques offers an effortless & effectual method for the calculable measurement of hemolysis; thus this method affords an assessment for the influence for different concentrations of biological molecules on the human red blood cells.

Some species of healing plants were used in the treatment of diabetes mellitus; a world-wide disease which affect huge amount of people therefore; Traditional plant & herbal preparations (e.g *Acacia Nilotica* & *Achillea Millefolium*) strengthly propose a normal basics to solve diabetic impediments and problems[8].

Acacia Nilotica; also called Gum Arabic tree; it is an aromatic spiny tree innate to Africa nurtures in South Africa; Egypt; Nigeria & Kenya; also it founds in Indian subcontinents [9]; It's a nitrogen fixing trees that propagates to 14-17 meter in the elevation & 2-3 m in thickness. African Zulu use the bark of *A. Nilotica* for the treatment of diarrhea; cough; leprosy & dysentery [10]. Kenyan people used the bark and root extracts as aphrodisiac for the remedy of sexual inability. The pod is used to treat tuberculosis [11]. where the powdered pods were consumed by Egyptian people for the treatment of diabetic man's [12]. In Northern Nigeria, the root is used as a Typically originate in Europe; Asia, & mild areas like North America; the *Achillea* genre; belong to the Asteraceae family; which is embodied by about 85 kinds [17]; *A. millefolium* (public yarrow) were used with many bids such as remedy; veterinary information & face paint [18].

The blossoming herbals have been described by having energizer; anti-spasmodic; vulnerary & diaphoretic actions, midst others, & for that is endorsed for the treatment of wintriness; bombasts; panic & rheumatic managements [19]. In the popular medicine; *Achillea Millefolium* is used for the handling of malaise; asthma; bronchitis, coughs; skin - inflammation; jaundice; diabetes; hepato-biliary maladies; menstrual ruling; gassiness; stomachache; hemorrhoids; dysmenorrhea & gastritis & also used up for its anti-tumor, anti-microbial, anti-inflammatory and anti-oxidant possessions [20, 21, 22].

remedy for malarial infections [13, 14]. *A. Nilotica* is opulent in many secondary metabolites, such as *Sapinines terpeneses*; tanninses; flavonoids; alkaloids & phenolics [15]. With a known pharmacological properties, thus making it relevant in the treatment of various ailments.

Although; *A. nilotica* produces tannins & alkaline compounds as a defensive method to remove mammals and insects from the plant; However, some of these alkaloids have been considered poisonous and some have affected the mental abilities of individuals exposed to this plant and excessive use leads to destruction of the liver and kidneys [16].

Materials and Methods

Plants samples

The current study included *Acacia Nilotica* & *Achillea Millefolium* which were obtained from a medicinal herb center; transferred to the laboratory; washed with distilled water, then dried on sterilized filter sheets at laboratory temperature, crushed & grinded by an electric mill & then stored in polyethylene bags in the refrigerator with (4°C) Until they are used for extraction.

Extraction of medical plants

The methanolic extraction of plants were studied using the Soxhlet extractor in the extraction process. The dilution ratio of methanol was 1:2 .

The extraction crude was collected followed by removing methanol from the extract; then the result crude was reduced to the concentrations to be studied. *Acacia Nilotica* concentrations were 10, 20, 50, 100, 250 mg/ml while *Achillea Millefolium* was 25, 50, 75, 100, 150, 200 mg/ml.

Samples

The study included the effect of *Acacia Nilotica* 10, 20, 50, 100, 250 mg/ml & *Achillea Millefolium* plant at a concentration of 25, 50, 75, 100, 150, 200 mg/ml on the hemolytic activity for both of them on blood samples from healthy people. It was ascertained that people did not take aspirin or any other drugs or factors effect on the proportions of blood components and their nature at least ten days before the blood withdrawal.

The hemolytic activity of the plants extracts was tested on the blood samples using optical spectrophotometry. 1 ml of blood was mixed with 1 ml of each plant extract with phosphate precipitate solution and raised for thirty minutes in 37°C; the mixture was centrifuged at 1500 rpm for ten minutes & then free hemoglobin concentration was measured by spectrophotometer at a wavelength of 540 nm. Distilled water and phosphate solution were used as calibrators. Each concentration was tested five times per sample for the whole samples respectively.

Then the hemolytic activity was calculated by using the following formula:

$$\% \text{ Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

At: absorbance of the sample

An: absorbance of the control (phosphate buffer)

Ac: absorbance of the control (distilled water)

The results then were analyzed by using Excel program 2010 .

Results and Discussion

For the importance of medicinal plants and their abundance of uses due to its rapid therapeutic effect; high efficiency; popularity & cheapness, it has been used in many fields, including cosmetics or as a treatment for many diseases without resorting to industrial chemical compounds [23].

Therefore *Acacia Nilotica* and *Achillea Millefolium*, which contain many medicinal and therapeutic compounds that protect and prevent tumors and many diseases, have been selected to know the side effects of these two plants [24].

The present results indicate that the two plants above have a clear effect in causing red blood cell decomposition. This effect increases as the concentration of extractors increases. These results were consistent with other results showing the analytical effect of the plant extract of acacia at the cellular level [25] and for the *Achillea Millefolium* as well [26].

Red blood cell decomposition was increased as the concentration of the above plant increased compared to red blood cell use with phosphate precipitate solution alone, with the highest degradation rate of 250mg/ml for Acacia spp. and 150 mg/ml for Achellia spp. plant.

The hemolytic activity of the blood cells is attributed to undetermined causes, including surface tension of the blood cell membranes, through analysis of the red blood cell plasma membrane due to increased dilution or fragmentation or due to osmolysis caused by increased membrane permeability [27], mainly due to the phenolic compounds such as flavonoids Phenolic acids. The presence of phenol increases the process of decomposition through the process of oxidation of hemoglobin, forming methmoglobin [28].

In addition, the presence of saponins in the plants is a major & important factor in the activity of hemolytic red blood cells by creating changes in the red blood cell membrane causing rupture followed by releasing of hemoglobin [29], and this can be useful in the removal of thrombosis that It is possible to occur after birth, as well as; it works to prevent the accumulation of thrombocytopenia and thrombosis through the effect on the level of calcium Ca, which contributes to the formation of blood clot during bleeding [30, 31].

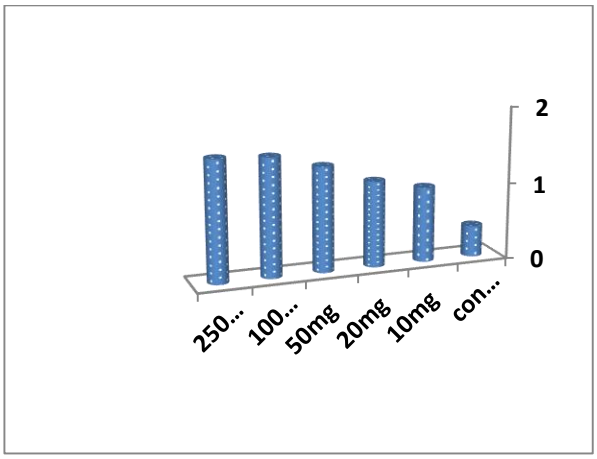

Figure (1): The effect of *Acacia Nilotica* on the hemolytic activity of RBCs.

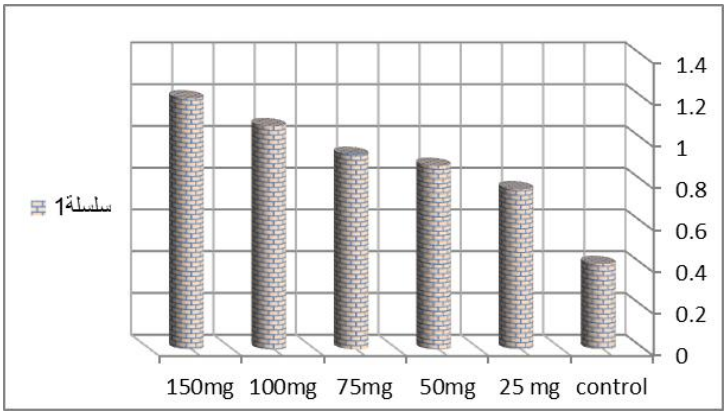

Figure (2): The effect of *Achillea Millifolium* on the hemolytic activity of RBCs.

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