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Anti-oxidant Activity, Brine Shrimp Lethality and Phytochemical Screening of *Blepharis linariifolia* Ethanolic Extract

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ABSTRACT

In this study, the ethanolic extract of *Blepharis linariifolia* (vernacular name in Sudan is *Al Saha*) was studied for its anti-oxidant activity, Brine shrimp lethality and phytochemical constituents. Phytochemical screening of this plant extract revealed the presence of alkaloids, tannins, flavonoids, saponins, sterols and triterpenes. The cytotoxic activity of the extract was conducted by using the brine shrimp assay. It was found that this plant extract has a wide safety margin (> 1000 µg/ ml) which was considered significant at $P \leq 0.05$. Study of the anti-oxidant activity of *Blepharis linariifolia* extract was conducted by using the radical scavenging method (DPPH). *Blepharis linariifolia* extract showed a remarkable anti-oxidant activity measured as 86 %.

Keywords: Radical Scavenging, Chemical Constituents, Brine Shrimp, *Blepharis linariifolia*

Introduction:

Medicinal plants are extensively utilized in traditional medicine for the treatment of different ailments. There is an increasing interest worldwide for the consumption of medicinal plants since they are inexpensive and widely available. According to the statistics of World Health Organization (WHO) more than 80 % of world population particularly in the under developed countries, provide their primary health care necessities from herbal resources [1]. Even in this modern time and even in developed countries, people still rely on traditional system of healthcare. It is believed that the use of native medicinal plants along with effective synthetic drugs is beneficial and can improve the quality of life and living standards of the native inhabitants [2,3].

Many plants are investigated for the development of active constituents which can be used as phytocompounds in drug development [4]. Extensive research in natural compounds from plants had a great contribution to the health care delivery system in many rural communities specially in Africa [5]. In the body, antioxidants act as free radical scavengers and thus protect cells from being exposed to free radicals and further cellular damage. This is the mechanism by which they protect the human body from several diseases attributed to the reactions of radicals. Numerous substances have been suggested to act as anti-oxidants and various phenolic antioxidants such as flavonoids, tannins, hydroxynaphthoquinones and coumarins have been shown to scavenge radicals. *Blepharis linariifolia* (Family: Acanthaceae) which is a native plant in Western parts of Sudan. is used as a forage for animals and responses to drought. In other African countries, it was found that *Blepharis linariifolia* (Family: Acanthaceae) a native plant in Western Sudan is used as a forage for animals and responses to drought, and in previous ethnobotanical studies it was stated to be used for the treatment of renal disorders by people in Western Sudan [6]. In other African countries, it was found that *Blepharis linariifolia* is used traditionally by the Maasai community in Kenya for the treatment of lung problems [7]. However, little is known about other biological activities of this plant. This study is an attempt to reveal the potentials of this plant in the treatment of diseases and explore its biological activities.

Materials and Method:

50 g of the whole plant ground powder of *Blepharis linariifolia* was soaked in 500 ml of 80 % ethanol for about twenty-four hours at room temperature. The extract was filtered through filter paper and the solvent was evaporated under reduced pressure using rotary evaporator apparatus. The percentage yield was found to be 32 %.

General phytochemical screening for the active constituents was carried out for *Blepharis linariifolia* ethanolic extract. Tannins were identified in the extract by the use of ferric chloride and gelatin salts. The sterols and triterpenes were tested by the use of acetic anhydride and Sulphuric acid. Akaloids were detected by Mayer's reagent and Valser's reagent. Flavonoids test was conducted by addition of 1% aluminum chloride solution Flavones were detected by 1% potassium hydroxide solution. Saponins were detected in the plant sample by placing 0.3 g of the extract in a test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, and taken as evidence for the presence of saponins. Test for cumarins was made by the use of 0.5N KOH and inspection under UV light. Test for Anthraquinone glycoside was conducted by 3% hydrogen peroxide solution [8].

Brine shrimp Lethality test was conducted by using a hatching tray (a rectangular dish of 22× 32 cm) half filled with filtered *Artemia salina* (shrimps eggs) solution. About 50 mg of eggs was incubated at 37º C. Then 20 mg of *Blepharis linariifolia* ethanolic extract was dissolved in 2 ml of ethanol. From this solution volumes of 5, 50 and 500 µl were transferred to vials (3 vials/ concentration). The solvent was allowed to evaporate over night. After two days of hatching and maturation, 10 larvae in each vial were pipetted and the volume was completed to 5 ml with sea water. This mixture was incubated at 25 º C for 24 hours under illumination. The vials were supplemented with ethanol and reference cytotoxic drugs to serve as negative and positive controls respectively. Data was analyzed with Finney computer program to determine LC_{50} values with 95 % confidence intervals [9].

Anti-oxidant Activity of *Blepharis linariifolia* was conducted by causing a DPPH radical scavenging assay which is used to test the anti-oxidant activity of *Blepharis linariifolia* ethanolic extract. In 96-wells plate, the ethanolic extract of *Blepharis linariifolia* allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The plant extract was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multi plate reader spectrophotometer. Percentage radical scavenging activity by the extract was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

$$\% \text{ inhibition} = \frac{A_{blank} - A_{sample} \times 100}{A_{blank}}$$

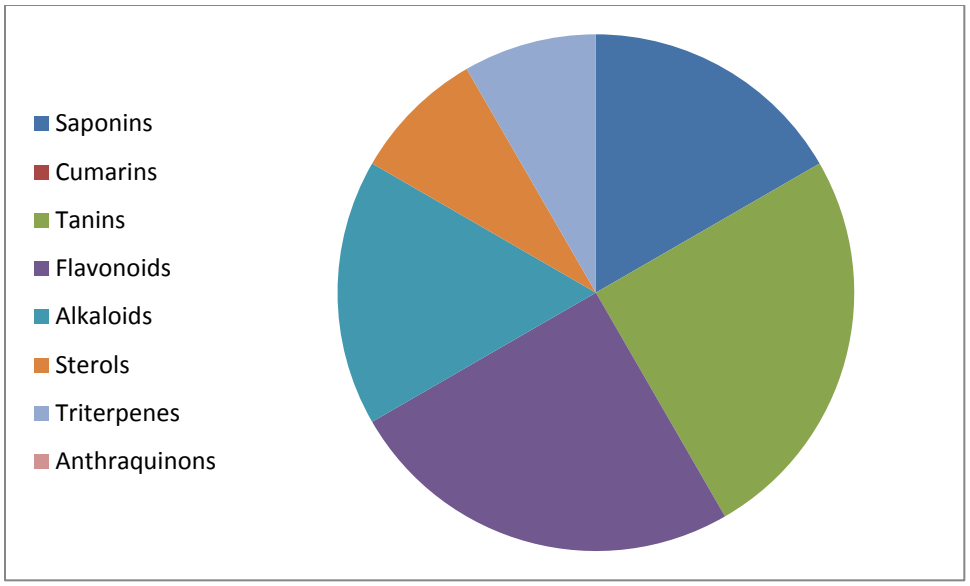
Where A_{blank} is the absorbance of the blank at zero time and A_{sample} is the absorbance of the sample after 30 minutes [10].

Statistical analysis:

All data for the radical scavenging activity were presented as means ± SEM (standard error of the means). Statistical analysis for this assay results was done using Microsoft Excel program, while the Brine shrimp lethality assay was analyzed by using Finny computer program [9].

Results and Discussion:

Phytochemical screening of ethanolic extract of *Blepharis linariifolia* was found to be positive for the presence of moderate amounts of alkaloids and saponins, high amounts of tannins and flavonoids, little amounts of sterols and triterpenes. However, it was found to be negative for the presence of cumarins and anthraquinones.



LC 50 of the ethanolic extract of *Blepharis linariifolia* was found to be 2090.705 µg/ml. Thus the extract considered to be safe. While, The percentage radical scavenging activity of *Blepharis linariifolia* ethanolic extract was found to be 86 %.

In spite the wide use of medicinal plants in health care systems, however, there is still a need for the elucidation of the potential medicinal effects of these plants as well as to ascertain their safety. Sudan is known to be rich in botanical flora used by natives for the treatment of various diseases. Kordofan, an area found in Western Sudan, is located between latitude 270 E and 300 E and people of these regions rely largely on traditional medicine practices and the use of medicinal herbs in the treatment of various diseases. There was no much scientific data found about the biological and/ or the chemical constituents of *Blepharis linariifolia*. However, a previous ethnobotanical study revealed that the whole plant of *Blepharis linariifolia* is used as a decoction in the folk medicine of Western Sudan for the treatment of urine retention [6] and the decoction of the fruits of *Blepharis linariifolia* is used for the treatment of stomach pains and kidney stones ([11]). In the present study, phytochemical screening of *Blepharis linariifolia* revealed the presence of flavonoids and alkaloids which may contribute to its usefulness as a biologically active medicinal. The high percentage of radical scavenging activity of this plant expresses its potency as an anti-oxidant. It may also considered as a safe plant since it had a weak cytotoxic activity when tested by using the Brine shrimp Lethality assay

according to the fact that the general toxic activity of plants extracts is considered weak when the LC 50 values are found > 1000 µg/ml [12]. Hence, *Blepharis linariifolia* can be seen as a potential source as an herbal remedy. Further studies will be carried out on *Blepharis linariifolia* in order to isolate, identify, characterize and elucidate the structure of its bioactive compounds

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