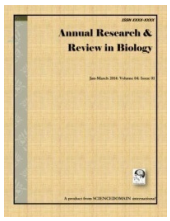

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Pharmacognostic and Wound Healing Studies of the Leaves of *Bassia eriophora* (Family: *Chenopodiaceae*) on Albino Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Objective: To study the pharmacognostic profile and wound healing effect of aerial parts of *Bassia eriophora* (Family: *Chenopodiaceae*), a wild plant in Saudi Arabia, on albino rats.
Methods: The microscopy, phytochemical and physiochemical evaluation of the *Bassia eriophora* aerial parts were carried out according to the standard procedure based on WHO guidelines. Toxicity and wound healing effects of this plant was performed to explore the medicinal values.
Results: Microscopically aerial parts of *Bassia eriophora* contains paracytics stomata, Long covering trichome, phloem fibers in a groups, spiny pollen grain, tannin containing cells, rosette crystals of calcium oxalate, spiral vessels, xylem vessels and fibers which serve as useful pharmacopoeial parameters for identifications. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, phenolic compounds, saponins terpens, tannins and flavonoids in alcohol extract. Physiochemical parameters such as total ash (20.62±0.30), moisture content (6.56±0.04) were revealed while distilled water extractive values was found more when compared with methanol extracts. The gel of *Bassia eriophora* was prepared and doses of 250 and 500 mg/kg were applied on excision wound. Significant reduction ($p \leq 0.001$) in wound size was observed.

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Conclusion: In the present studies pharmacognostics parameters of Bassia plants was established and pharmacologically it showed a non toxic and the use of gel containing *Bassia eriophora* extract as a wound-healing agent.

Keywords: Microscopy; phytochemical; physiochemical; toxicity; gel formulation; WHO guidelines.

1. INTRODUCTION

Bassia eriophora (Family: *Chenopodiaceae*) is a common herb growing in Saudi Arab, commonly known as ummulhas, gteena, alguteen. The related plant is used in folk medicine to treat renal and rheumatic diseases [1]. Reviewing current literature showed that only a few species of Bassia have been investigated and were found to contain triterpenoidal saponins identified as bassic acid glycosides [2]. From the aerial parts of *Bassia muricata*, two acylated flavonoid glycosides as well as four known triterpenoidal saponins were isolated [3]. Few species of Bassia showed toxic effects on grazing animals [4]. Some of Bassia species showed the antimicrobial and antirheumatic activities [1]. The wound-healing process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution. For a proper wound to heal, all the above four phases must occur in the proper sequence and time frame [5]. Skin is one of the most readily accessible organs on human body for topical administration and is the main route for topical drug delivery system. Gel is one of the tropical formulations, penetrates into deeper area of skin, absorbed by blood and transported to the site of action where inflammation occurs [6]. The available literature revealed that no pharmacognostic, toxicity and wound healing studies have been carried out on *Bassia eriophora* aerial parts. Hence the present analysis was under taken to investigate microscopic, phytochemicals, physiochemicals for established to identification while toxicity and wound healing for safety and pharmacological properties.

2. MATERIALS AND METHODS

2.1 Chemicals

Polyethylene glycol, Glycerin, triethanolamine and all other chemicals were obtained from Merck & Co. Inc (USA). Carbapol-934 was supplied by Sigma (USA) and Betadine® (10% w/w Povidone-Iodine) local market.

2.2 Collection and Authentication of Plant

Bassiaeriophora aerial parts were collected in March, 2012 from Al-Kharj region of Saudi Arabia and were authenticated by taxonomist (Dr. M. Atiqur Rahman), of the College of Pharmacy, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), K.S.U, Riyadh. A voucher specimen of this plant has been deposited at the herbarium of College of Pharmacy, Salman Bin Abdulaziz University, Al-Kharj, and Kingdom of Saudi Arabia.

3. PHARMACOGNOSTICS STUDIES

The aerial parts of the plant was properly dried and placed into a large mortar and pounded. The powdered sample was stored in airtight container for pharmacognostic studies.

3.1 Microscopic Examinations

Sections of the powdered sample were prepared for the microscopic studies. The standard laboratory methods were used for staining [7]. The most prominent characters were identified using computerized compound microscope.

3.2 Preliminary Phytochemical Study

A phytochemical screening was conducted on aerial parts of *Bassia eriophora extracts* using standard qualitative methods to confirm the presence of phytoconstituent. Preliminary phytochemical analysis of hydro alcoholic extract was done according to previous methods [8,9].

3.3 Physicochemical Study

For the moisture content 2 grams of raisins pulp were spread out in a tarred stainless capsule and then dried in a drying oven at 80℃ until constant weight was reached. The moisture content was calculated from difference in weight according to method described [10]. Ash Values were determined according to [11], and extractive value was determined according to previous method [12].

4. ANIMAL STUDY

4.1 Preparation of Extracts

The air dried aerialparts of Bassia *eriophora* were powdered using grinder. The powder (200 g) was extracted with 2000ml of 90% ethanol in a percolator till color of solvent in percolator is nearly equal as solvents then the solvent was filtered by using whatman-1 filter paper. The obtained extract was concentrated to under reduced pressure at 45℃. The thick solution of extract was lyophilized by means of freeze drying. The obtained dry powdered extract (26.5g) was used for experimental studies.

4.2 Collection and Preparation of Animals

Albino Wistar rats and Swiss mice of both sex were procured from the animal house, college of Pharmacy, Salman Bin Abdulaziz University, Al-kharj, Saudi Arabia. The protocol for the study was permitted by the Ethical Committee of the college of Pharmacy, Salman Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia. All animals were house under standard laboratory conditions at room temperature of 22±2℃, humidity (55%) and were exposed to 12h light/dark cycle fed on standard chow diet and water.

4.3 Acute Toxicity Test

The acute toxicity test of ethanol extract of *Bassia eriophora* was determined according to the method illustrate by Tanko et al. [13]. Thirty (30) mice were divided into five groups (n=6) and test extract was administered orally at the doses of 50 to 3000mg/kg body weight. Signs of acute toxicity and number of death per dose within 24h were recorded.

4.4 Preparation of Topical Formulations

Control base, 1% and 2% gel were separately prepared using *Bassia eriophora* extract according to method Khan et al. [14]. Carbapol-934 (0.5g) was mixed with an adequate amount of distilled water in three different beakers and kept in an oven at 100℃ for 20 min to obtain a homogenous viscous mixture, and then cooled to room temperature with continuous stirring. Triethanolamine-10ml was added drop-wise with continuous stirring using mechanical stirrer. A weighed amount of the extract was added to the beakers and mixed using glass rod. Other ingredients dimethyl sulfoxide (DMSO) 10ml, polyethylene glycol (PEG-200) 1.5g, polyethylene glycol (PEG-400) (1.5g) was added with constant stirring to prepare 100 gm gel formulation.

4.5 Wound Healing Test

The excision wound model was used to monitor wound contraction and wound closure time [15]. Four groups of albino rats were used in the experiment, 5 animals each. At the beginning of the experiment, the dorsal skin was trimmed with an electric clipper. After 24 hours, all animals were anesthetized with diethyl ether and the shaved areas were sterilized with 70% alcoholic solution. A predetermined dorsal area (approximately 2.5cm^2) was excised using toothed forceps, scalpel and pointed scissors. A fresh surgical cutting edge was used for the perpendicular cut in each animal and during the operation the tension of skin was kept constant.

Wound of the first (normal control) and second (positive control) groups were treated with the base gel and Betadine, respectively. Animals of the third and fourth groups were treated with 1% and 2% *Bassia eriophora* extract gel respectively. Wounds were fully covered topically with the base gel, standard drug and the extract gels on the wound surface once a day for 20 days. The wound size was determined with the help of *vernier caliper* immediately after the wound excision at every 4 days until healing was accomplished. The reduction in the wound size was calculated according to the following formula:

Wound contraction (%) = (DWi-DWt) /DWi × 100;

Where: DWi = the wound area immediately after wound excision, DWt = = the wound area on day t. Small portions of healing area were cut and keep in formalin solution for photomicrograph study.

4.6 Statistical Analysis

All data were representative in triplicate and presented as mean ± S.E.M. The differences between groups were study by Student's t-test and oneway ANOVA using Graph-Pad Prism 5 software.

5. RESULTS

5.1 Powder Microscopic Characteristics

The powder plant material is greenish in color with white wool like constitution, showing epidermal cell along with stomata (Fig. 1A), long or fragments of unicellular or multicellular covering trichomes (Fig. 1B), lignified fibers (Fig. 1C), Pollen grain (Fig. 1D), Tannin containing cells (Fig. 1E), Rosette crystals of calcium oxalate (Fig. 1F), spiral vessels having simple pits (Fig. 1G), Xylem vessels and fibers (Fig. 1H)

5.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening of aerial parts of *Bassiaeriophora mainly* revealed the presence of saponins, triterpenoids, tannins and flavonoids (Table 1).

5.3 Physicochemical Parameter

Physicochemical analysis of powder Total ash value (20.62±0.30), Acid insoluble ash (3.07±0.05) and water soluble ash (12.77±0.03). Percentage moisture content of *Bassia eriophora* was 6.56±0.04 while percentage extractive value in distilled water was highest (14±0.05) and in hexane was lowest 2.35±0.05 (Table 2).

5.4 Toxicity Study

The results indicated that up to 3000mg kg^{-1} dose of *Bassia eriophora* extract did not produced any symptoms of acute toxicity.

5.5 Excised Wound Healing Effect

The observed percentage excised wound contraction with base gel, Betadine, 1% Gel and 2% Gel were 9.26±1.43, 11.27±1.13, 11.39±1.05 and 13±1.46 on 4^{th} days while 56.70±2.30, 60.25±0.25, 78.68±2.69 and 84.21±1.79 on end of 20 days (Table 3). The photographic effect of wound healing was shown in Fig. 2 while photomicrograph of skin section was shown in Fig. 3.

Table 1. Phytochemical present in ethanolic extract of aerial parts of *Bassia eriophora*

Phytoconstituent	Test	Result
Alkaloids	Mayer's test	-
	Wagner's test	-
	Dragendroff's test	-
Carbohydrates	Molisch's test	+
	Benedict's test	-
	Fehling's test	+
Glycosides	modified Borntrager's test	-
	Keller-Killiani test	-
Saponins	Froth test	+
	Foam test	+
Steroids and Terpenes:	Salkowski's test	+
	Liebermann Burchard's test	+
Fats & oils	Tshugajeu test	+
Resins	Stain test	-
Phenols & Tannins	Acetone-water test	+
Flavonoids	Ferric Chloride test	+
	Alkaline Reagent test	+
Proteins & Aminoacids	Lead acetate Test	+
	Shinoda test	+
	Xanthoproteic Test	+
	Ninhydrin Test	+
Diterpenes	Biuret Test	+
	Copper acetate Test	+

Table 2. Physiochemical properties of ethanolic extract of aerial parts of *Bassia eriophora*

Parameter	Result	
Ash value	Total ash	20.62±0.30
	Acid insoluble ash	3.07±0.05
	Water soluble ash	12.77±0.03
Percentage moisture content	Moisture content	6.56±0.04
Percentage extractive value	Hexane	2.35±0.05
	Chloroform,	5.2±0.05
	Methanol	10.33±0.11
	Distilled water	14±0.05

n = 3, mean ± SEM

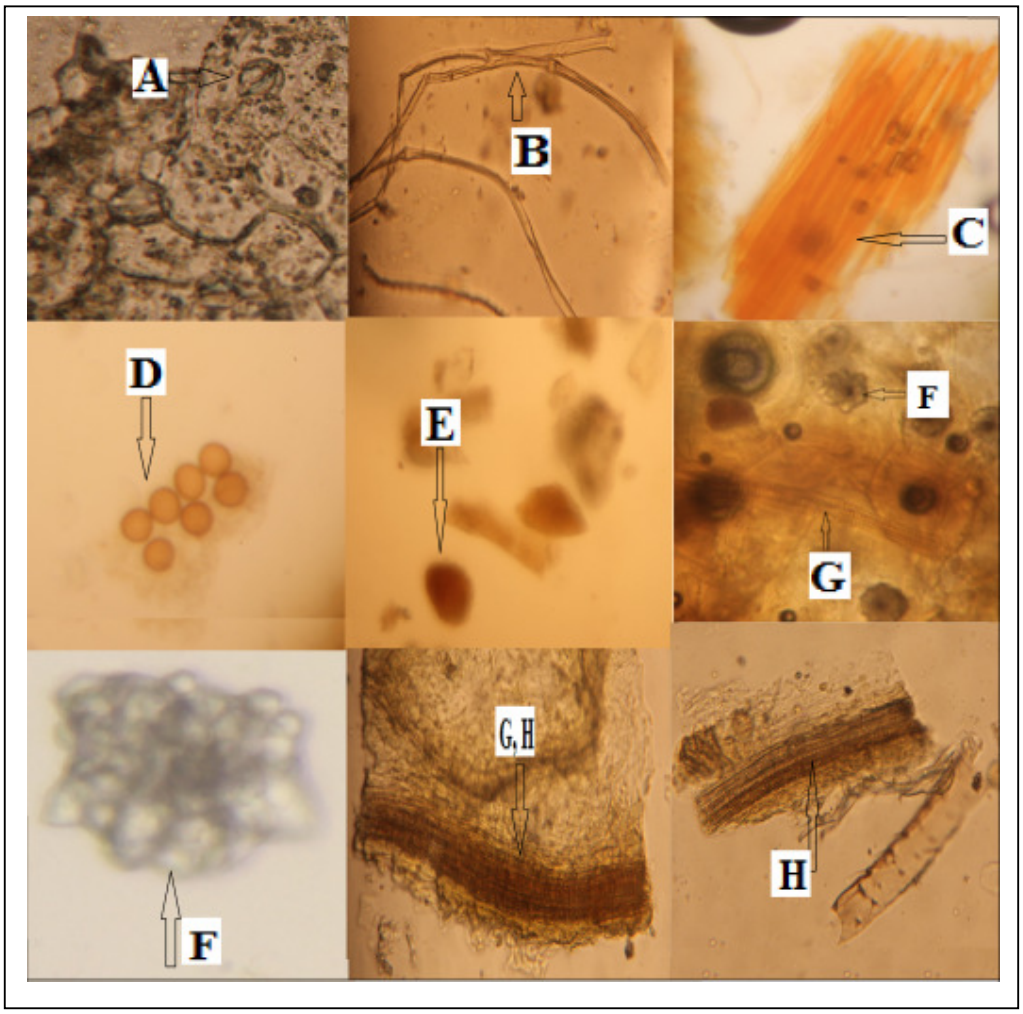

Fig. 1. Powder microscopy study (x40) of aerial parts of *Bassia eriophora* (A: Epidermis cell with stomata, B: Long covering trichome, C: fibers, D: pollen grain, E; Tannin containing cells, F: Rosette crystals of calcium oxalate, G: Spiral vessels, H: Xylem vessels and fibers)

Table 3. Excision wound studies showing percentage reduction in wound size, when treated with base control and gel formulations

Treatment	4th Day	8th Day	12th Day	16th Day	20th Day
Base gel	9.26 ±1.43	17.88±1.32	31.19±0.89	45.26±1.76	56.70±2.30
Betadine	11.27±1.13**	21.4±1.33**	32.37±1.22**	47.27±1.93**	60.25±0.25 **
1% Gel	11.39±1.05**	21.24±1.5**	34.26±2.24**	57.21±2.06***	78.68±2.69 ***
2% Gel	13±1.46***	25.44±2.39***	45.62±2.86***	65.13±1.08***	84.21±1.79 ***

*n = 5, mean ± SEM. Values are the average of triplicate experiment. **p<0.01, ***p<0.001 when compared to basal base*

6. DISCUSSION

The aerial parts of *Bassia eriophora* were used by local people without standardization. The pharmacognostics standardization of a crude drug is an important part for establishing its correct identity. Pharmacognostic parameters must be established before any crude drug is included in an herbal pharmacopoeia. Microscopic method is one of the simplest and cheapest methods for the correct identity of the source materials [16,17].

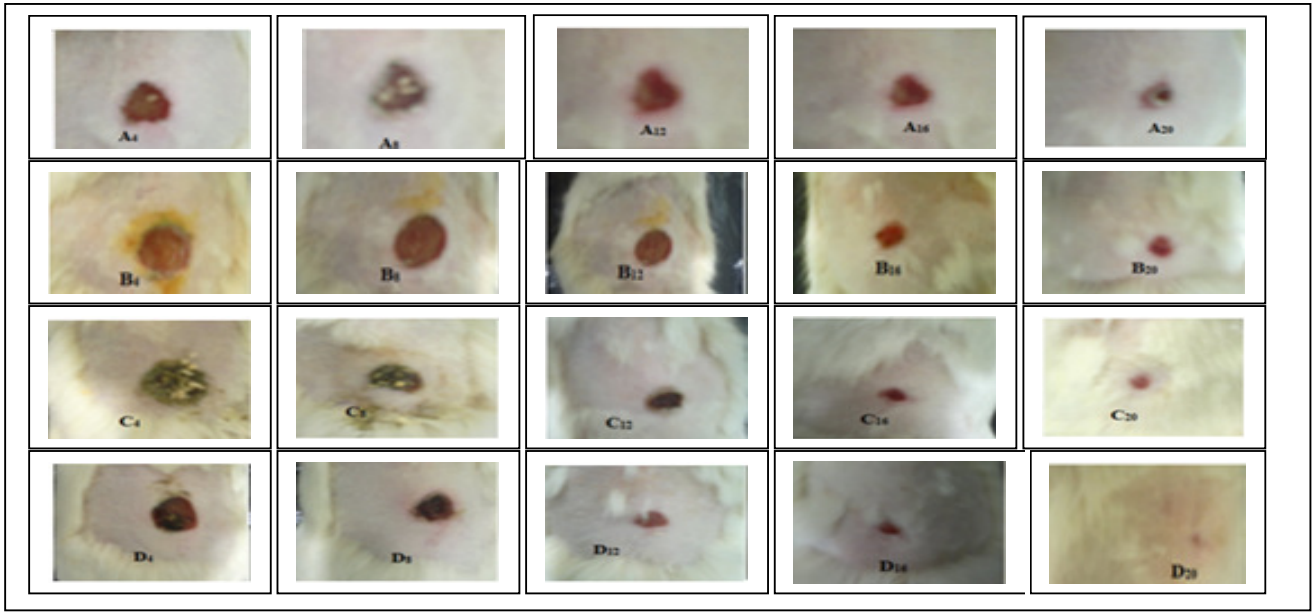

Fig. 2. Excision wound model of rats (4, 8, 12, 16 and 20 days) showing various phases of wound healing. A: Base gel; B: Betadine; C: Bassia (1% w/w); D: Bassia (2% w/w)

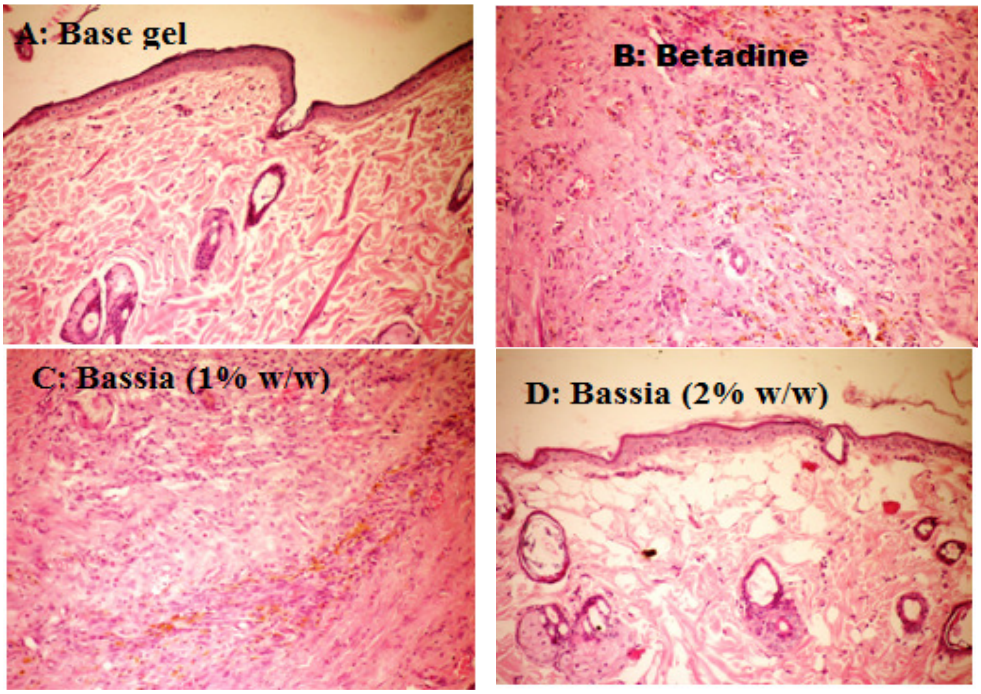

Fig. 3. Photomicrograph of skin of rats showing various histopathological changes. A: Photomicrograph of skin of basal gel group showing normal epidermal and epidermal layer,B: Photomicrograph of skin of Betadine group showing vascularized granulation tissue.C: Photomicrograph of skin of Bassia 1% gel group showing massive granulation tissue formation. D: Photomicrograph of skin from Bassia 2% gel group showing normal histological layers

The pharmacognostic standards of aerial parts of *Bassia eriophora* are carried out for the first time in this study. Microscopical studies indicated the presence of characteristics stomata, trichomes, pollen grain, fibers, vascular bundles and ca-oxalate crystal. These macroscopical characters of the aerial parts can serve as diagnostic parameters. Preliminary phytochemical screening of aerial parts of *Bassia eriophora* powder showed the presence of carbohydrates, saponins, terpenoids, tannins, flavonoids, proteins and amino acids. Main attraction of phytochemical screening was presence of tannins terpenoids and flavanoids. They were known to show medicinal activity. Ash values, moisture content and extractive values can be used as reliable aid for detecting adulteration. These studies help in the identification of the plant materials [18]. Percentage extractives and ash analysis were carried out and results showed that water soluble ash was higher than acid insoluble ash. The water extractive value of aerial parts was higher than methanol, chloroform and hexane Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. Extractive values are also helpful to evaluate the chemical constituents present in the crude drug and also assist in estimation of specific constituents soluble in particular solvents [19,20]. The extract of *Bassia eriophora* did not produce any symptoms of acute toxicity in present study. Furthermore wound healing and photomicrograph of skin confirm the safe use of this plant. The wound is attack by microbes when expose to the external environment [21]. If a wound becomes infected, the acute phase of inflammation becomes pronounced leading to further production of tissues oxidants which damage cellular membranes, DNA, proteins, lipid and extracellular matrix [22]. Wound healing is a dynamic and complex process of restoring cellular structures in injured tissue to its normal state. The wound contraction is a process that occurs during the healing process, beginning in the fibroblastic stage. In the final phase of wound healing, the wound undergoes contraction resulting in a small scar of tissue. The free radicals which were generated at the site of injury may protect the wound from invasion by

microbes [23]. Treatment of the excision wounds with the different gel formulations of the aerial parts of *Bassia eriophora* gave good signs of the wound healing potency of the plant similar to the Betadine a standard drug. It was observed that the wound contracting ability of the formulated gels were significantly greater than that of the control. Both the tested gel of *B. eriophora* extracts showed a significant wound healing from the 4th days and onwards, which was comparatively higher compared to the standard drug. The percentage wound contraction was much more with the 2% Gel indicated that the plant extract produced a dose dependent effect. The photomicrograph of skin of rats also confirms the healing ability of aerial parts of *Bassia eriophora.* Tannins, the main components of many plant extracts, act as free radical scavengers. The preliminary phytochemical study showed the presence of antioxidant such as tannins and other phenolic compounds. It has reported that antioxidant enhance wound healing process throughout due to free radical scavenging action [24,25]. However, further studies are needed to isolate the active compounds responsible for wound healing activity.

7. CONCLUSION

The pharmacognostics standardization of *Bassia eriophora* was recognized the safe with significantly attributed to healing potential in excision wound. The main mechanism behind the significant healing potential is antioxidants phytochemicals. Further, more phytochemicals pharmacological screening is required to investigate the correct mechanism.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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