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In Vitro Activity of Hypoglycemic Plant *Barleria noctiflora* with Wound Healing and Antioxidant Properties

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Abstract:- In order to test the antioxidant activity of the chosen plant extracts, multiple techniques were used. The purpose of this research aims to assess the qualities and treatment of diabetic patients in our nation. In order to distinguish the bioactive compounds and make their hypoglycemic effects clear, the study demonstrates the importance and focus on medicinal herbal plants. A medication derived from medicinal plants was crucial in addressing diabetes. The primary groups of anti-diabetic herbs now used in traditional medicine include Gymnema sylvastro, Allium cepa, Allium sativum, Aloe vera, Azadirachta indica, Syzygium cumini, etc.

Keywords:- *Barleria noctiflora, Anti-Diabetic, Wound Healing, Antioxidant.*

I. INTRODUCTION

➢ *Diabetes*

Hyperglycaemia and glucose intolerance are the hallmarks of diabetes mellitus, which is defined as a disorder, a group of illnesses caused by insulin shortage, decreased insulin action, or any mix of the two. The natural ingredient of insulin, which is endogenous, regulates blood glucose levels. When the amount of systemic blood glucose increased, the endogenous pancreas produced insulin to control the blood sugar. Diabetes mellitus' effects can cause renal failure, nerve damage, and eye impairment. These outcomes include minor vascular injury, also known as microvascular disease. The vascular arteries are rapidly becoming harder and narrower, which increases the risk of stroke and coronary artery illness. Many medicinal plants have been provided a potential source of anti-diabetic principles and are widely used for the treatment of diabetes mellitus in various traditional systems of medicine worldwide and many of them are known to be effective against diabetes (Reddy et al., 2005). [1, 2] The anti-diabetic drugs from plants in current clinical use and their similar mechanism of action of herbal components are preferred mainly due to lesser side effect and low cost. Conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. In addition to adverse effects, drug treatments are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences (Prabhakar and Doble, 2011) [3]

➢ *Objective:*

To test the powerful live plant fractions wound-healing & anti-diabetic properties.

➢ *Wound Healing*

Skin injury triggers processes that renew their function in defending the person from dangerous external elements. Diabetes causes delayed injuries, and even with enough and approximate treatment, non-curative wounds can linger for weeks. Such wounds are challenging to treat. Confirmatory research using both human and animal models indicates a number of anomalies in the different stages of healing of wounds.[4]

II. PLANT PROFILE

- Hindi Name: Arusa
- Common Name: Night Blooming *Barleria*
- Family: *Acanthaceae* (Acanthus Family)
- Synonyms: *Barleria Ramose*, *Barleria canthus noctiflora*
- Flowering: December-February.
- Medicinal uses: Night-Blooming *Barleria* is being widely used as Folk and ayurvedic medicine, e.g., in treatment of diabetes.
- Night-Blooming *Barleria* is a very rare plalnt, found in the scrub jungles in South India and Sri Lanka.

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Fig 1 Barleria Noctiflora Plant Photo

III. MATERIAL AND METHODS

➢ *Collection of Plant Materials:*

Barleria noctiflora was collected during the winter season.[5]

➢ *Extraction:*[6,7]

- *Successive Extraction:*

Using the Soxhlet apparatus, 500g of the powdered aerial portion of *Barleria noctiflora* were consecutively extracted for 72 hours with petroleum ether, chloroform, and ethanol. In a rotavapor, the extracts were concentrated till dry. The extract's yield was calculated. Until their next usage, all of the extracts were kept safe at 4°C in a refrigerator.

- *Extraction of crude:*

Barleria noctiflora aerial part powder weighing 500g (500 gm) was extracted with Methanol in a Soxhlet system for 72 hours. 55.3g (11.06% w/w) of greenish brown solid were produced. After maturation for seven days, the dry powder (50 gm) was extracted with water (250 ml) and concentrated to produce a greenish-brown solid. In a rotavapor at a regulated temperature (40–50 °C) and lowered pressure, all the extracts were concentrated to dryness. Until their next usage, all of the extracts were kept in a refrigerator at 4°C.

- *Fraction:*

200 cc of water and the dehydrated crude Methanolic extracts were added to a stoppered flask, and mechanically agitated for one to two hours in a flask shaker. Water did not entirely dissolve the ethanol extract. Filtration was used to separate the Methanol extract's water-insoluble component, and the same technique was used to further fractionate it using Methyl acetate and n-butanol. The retrieved supernatants from the originally stated fraction were condensed, dried by evaporation, and their yield in % was calculated.

IV. EXPERIMENTAL DESIGN

➢ *In Vitro Antioxidant Studies of Extract:*

In vitro antioxidant tests were carried out to compare the antioxidant activity of the plant extracts. For *in vivo* experiments, the extracts with the best antioxidant properties were chosen. Following the steps outlined below, the various plant extracts' scavenging abilities against various radicals were tested. The absorbance was determined in comparison to a blank solution in each experiment. Neither an extract nor a normal addition were used in the control. The extracts or standard had final concentrations of 1000, 500, 250, 100, 50, 25, 10, and 5 g/ml. The IC50, or the level at which a sample will salvage 50% of free - radical, was used to express the findings of all *in vitro* antioxidant scavenging activities.

Different extractions of *Barleria noctiflora*, including Petroleum ether extract (PEBN), chloroform extract (CEBN), methanolic extract (MEEBN), crude methanolic extract (CMEBN), and aqueous extract (BNE), were used to study the *in vitro* antioxidant activity (AEBN).[8]

- *DPPH Radical Scavenging Activity:*

The DPPH test is complemented by the assessment of antioxidants' capacity to scavenge DPPH radical stability. 97 In methanol, the free radical DPPH has a purple hue. When it interacts with a hydrogen donor, it is reduced to the equivalent hydrazine, which has a yellow colour. Separately, the liquid was fully combined before being left in the dark for an hour. To create the control sample, 2 ml of DPPH and 1 ml of methanol were combined. 98 A spectrophotometer was used to measure the absorbance at 517 nm. Three repeat measurements of the samples were made.[9]

- *ABTS-Radical Scavenging Method:*

The radical cation of ABTS+ exhibits an absorbance in the ABTS test that possesses the 734 nm wavelength signature. In this test, the ABTS radical cation, which is a blue-green chromogen, is used to create the radical in a stable state. The %age inhibition of absorbance at 734 nm is then computed when the produced coloured Inside the reaction mixture, radical and antioxidant are mixed and transformed back into colourless ABTS. The reaction between 2.4 mM potassium persulfate solution and 7 mM

ABTS solution (1:1) created the radicals of the ABTS+ cation, which were then kept at room temperature for 12–16 hours before usage in a dark environment. To get an absorbance of 0.7 at 734 nm, 100 ABTS+ solution was diluted with methanol. The absorbance was measured 30 minutes after the addition of 3 ml of diluted ABTS+ solution, 30 l of standard ascorbic acid (5-1000 g/ml), and 30 l of plant extract. Each assay included an appropriate blank run. The reading was done in duplicate for every page. Using the formula from the prior experiment, the absorbance of % inhibition at 734 nm was determined.

- *Wound-Healing Activity:*

Reactive oxygen species (ROS), which have negative effects on cells and tissues, are generally acknowledged to be damaging to wound healing. It has been demonstrated that topical treatments of substances with free radical scavenging activities in patients promote wound healing and shield tissues from oxidative damage. Studying the percentage of ethyl acetate of *Barleria noctiflora's* ability to heal wounds was of interest due to its *in vitro* antioxidant activity and ethno medical data.[10]

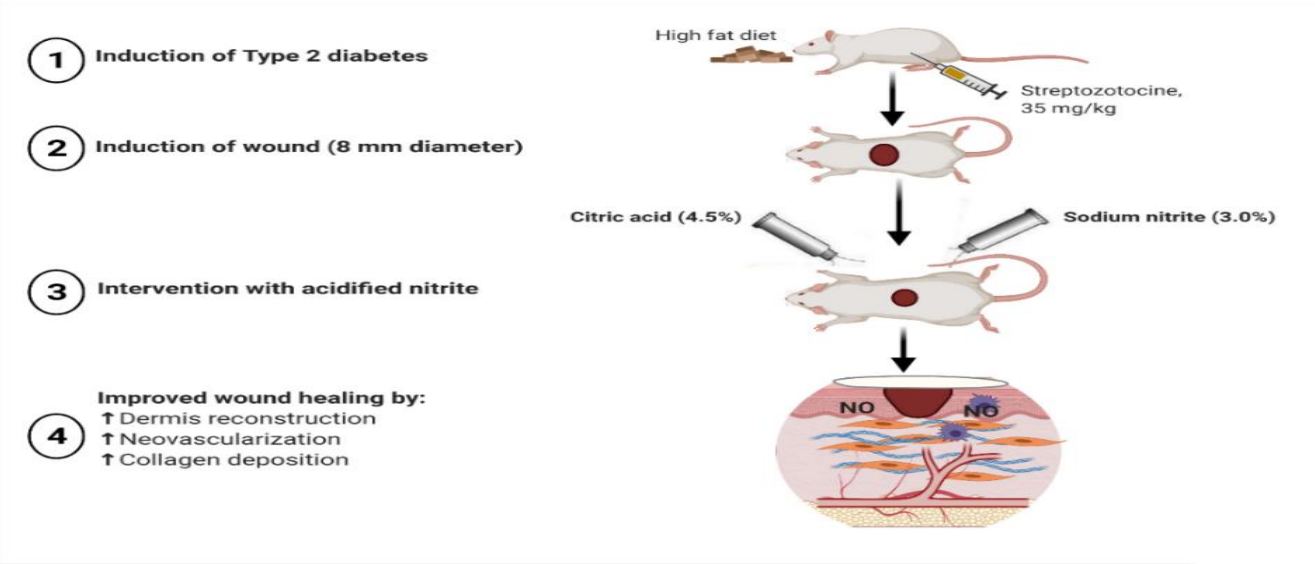

Fig 2 Wound Healing Rat's Model

V. RESULT AND DISCUSSION

➢ *In vitro Antioxidant Studies of Extracts:*

Different extracts of *Barleria noctiflora* in pet ether (PEBN*), Barleria noctiflora* in chloroform (CEBN), *Barleria noctiflora* in methanol (MEBN), *Barleria noctiflora* in ethanol (EEBN), *Barleria noctiflora* in crude ethanolic extract (CEEBN), and *Barleria noctiflora* in aqueous extract were used to test the in vitro antioxidant activity (AEBN).

- *DPPH Radical Scavenging Activity*[11]

Barleria noctiflora's methanolic extract showed significant antioxidant activity using the DPPH technique. The DPPH is a stable free radical that is frequently used to evaluate the capacity of antioxidants to scavenge free radicals. A stable free radical called DPPH may take a hydrogen ion or an electron to change into something like a permanent diamagnetic compound. The drop in absorbance at 517 nm brought on by antioxidants serves as a measure of the DPPH radical's capacity for reduction. The crude ethanol extract outperformed the other extracts in terms of its ability to scavenge DPPH radicals and perform like the reference standard. From the results, we can see that the DPPH radical scavenging activity has a dose-dependent relationship.

Table 1 Barleria Noctiflora's in Vitro Antioxidant Potential

Extract	IC_{50} values ± SE *					
	DPPH	**ABTS**	**H_2O_2**	**Lipid peroxidation**	***p*-NDA**	**AlkalineDMSO**
Pet Ether	367.16±1.93	271.4±0.51	268.03±0.05	336.16±1.34	330.43±3.43	349.03±0.68
Chloroform	304.2±3.75	254.03±0.33	256.9±0.90	204.63±0.08	186.06±0.66	264.73±0.23
Successive Ethanol	214.5±1.15	166.73±2.05	196.9±0.10	179.23±0.12	147.4±0.25	166.36±0.12
Crude Methanol	202.36±2.67	128.33±0.44	174.76±1.08	152.23±0.74	146.76±0.44	151.11±0.05
Crude Aqueous	256.73±1.82	210.86±0.27	236.26±0.20	195.06±0.14	176.2±0.30	181.03±0.51
Standard	200.46±2.79	106.6±0.17	151.83±0.12	132.03±0.06	136.73±0.27	139.76±0.66

* Three measurements' average; data are given as mean and SEM

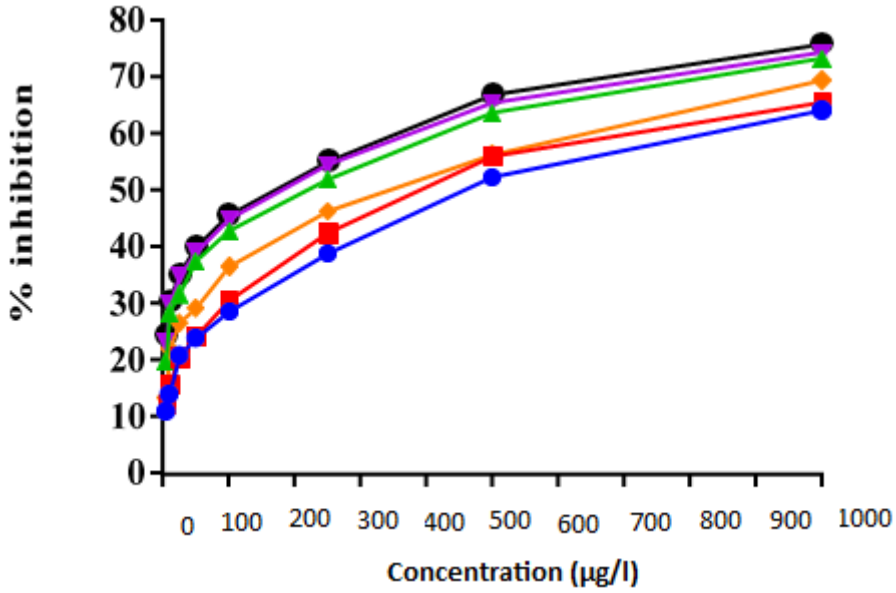

Fig 3 Activity to Scavenge DPPH Radicals

- *Radical Scavenging Method using ABTS:*[12]

All of the extracts showed robust, concentration-dependent radical scavenging activity in the ABTS method. The IC50 values were discovered to be 271.40.51, 254.030.33, 166.732.05, 128.330.44, 210.860.27, and 106.60.17 for standard ascorbic acid, respectively (Table 1 & Figure 4). In order to screen complex antioxidant combinations such plant extracts, drinks, and bodily fluids, Radical scavenging activity in ABTS varies., which entails a more extreme, chemically manufactured radical. The estimation of antioxidant activity using ABTS has attracted interest due to its versatility in both organic and aqueous environments as well as its stability over a wide pH range. As a result, the extract's ability to neutralise free radicals demonstrated a direct contribution from its phenolic components.

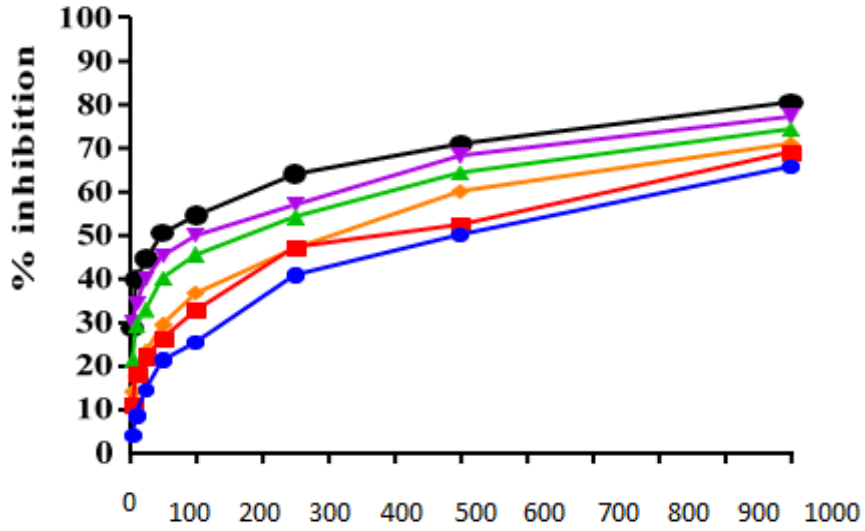

Fig 4 Radical Scavenging Method using ABTS

- *Wound-Healing Activity:*

In excision wound healing experiments, MAFBN and the common medicine nitrofurazone showed significant activity, comparable to the diabetic control. Over the course of 16 days, significant (P 0.01) %age shrinkage of the excision wound area was 96.6 0.10 and 95.8 0.27 in the MAFBN groups, compared to 99 0.25 in the nitrofurazone standard group (Table-2 & Figure-5). In the diabetic control group, wound %age contraction was significantly delayed compared to the normal control group. Because full epithelization was noticed on the same day of the MAFBN (10% w/w) and routine medication, the length of epithelization in diabetic control rats was 5 days longer than in normal control rats (18.83 0.16).[13,14]

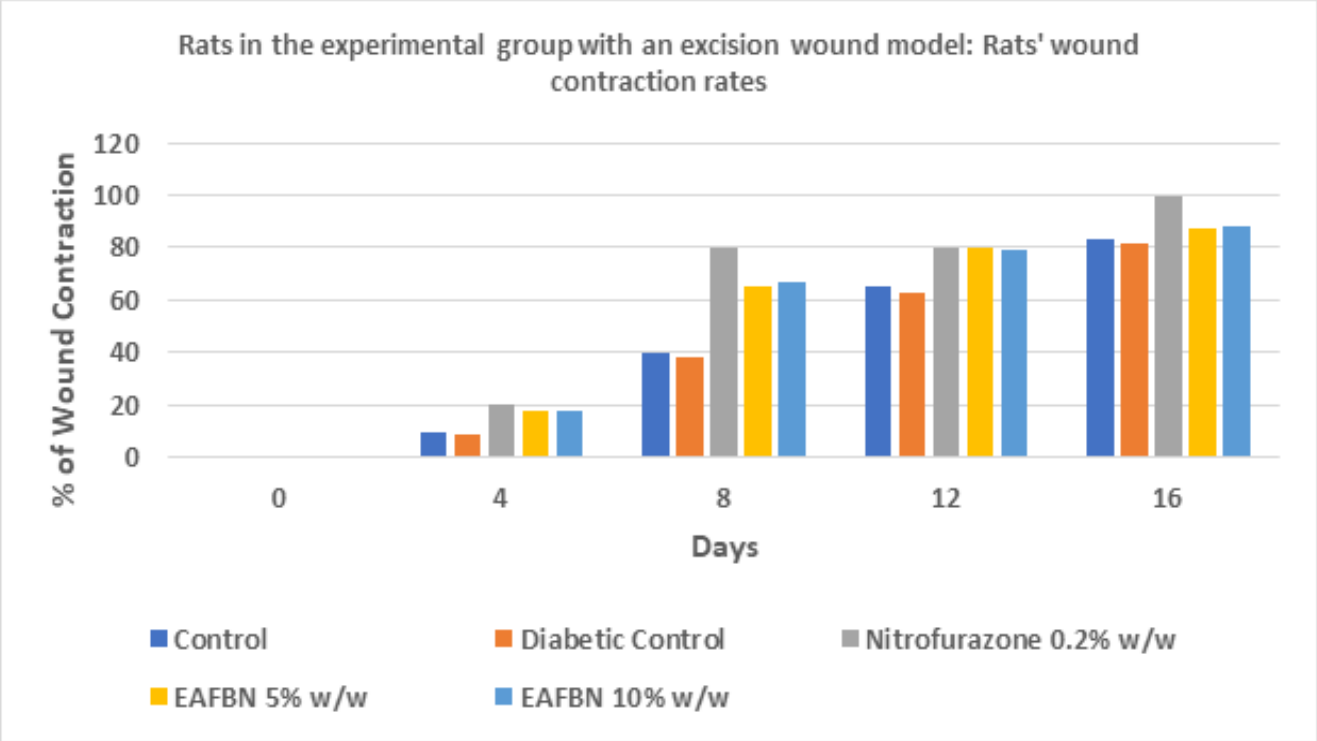

Fig 5 Rats in the Experimental Group with an Excision Wound Model: Rats' Wound Contraction Rates.

Table 2 Rats in the Experimental Group with an Excision Wound Model: Rats' Wound Contraction Rates.

Treatment	%Age of area of wound contraction				Period of Epithelialization (days)
	4th day	8th day	12thday	16thday	
Control	15.25±0.07	42.76±0.07	71.82±0.08	92.1±0.27	18.81±0.16
Diabetic control	13.21±0.35**	40.14±0.40**	69.71±0.33**	86.83±0.27**	23.62±0.55**
Nitrofurazone 0.2% w/w	24.71±0.14**	76.73±0.42**	86.42±0.50**	97±0.25**	15.15±0.16**
EAFBN 5% w/w	17.5±0.42**	64.1 ±0.34**	87.0±0.55**	92.8±0.27**	15.5±0.34**
EAFBN 10% w/w	12.5±0.18**	65±0.32**	82.5±0.23**	97.6±0.10**	15.73±0.16**

In the incision wound model, the skin breaking strength (gm) of 290.83 3.0 was considerably higher in the animals administered with topically MAFBN 5% w/w compared to MAFBN 10% w/w and normal care treatment both showed approximately the same significant skin breaking strength. However, it was lower in diabetes control and normal control (Table -3 & Figure-6).

Table 3 The Consequences of MAFBN on the Shattering Strength of Just an Experimental Group of Rats in an Incision Wound Model

Treatment	Breaking strength (gm)
Control	217.5±3.81
Diabetic control	199.33±3.94**
Nitrofurazone 0.2%w/w	350±2.88**
MAFBN 5% w/w	290.83±3.0**
MAFBN 10%w/w	342.5±3.81**

Table 4 The Consequences of MAFBN on the Shattering Strength of Just an Experimental Group of Rats in an Incision Wound Model

TREATMENT	Breaking strength (gm)
Control	217.5±3.81
Diabetic control	199.33±3.94**
Nitrofurazone 0.2%w/w	350±2.88**
MAFBN 5% w/w	290.83±3.0**
MAFBN 10%w/w	342.5±3.81**

➢ *Column Chromatography was used to isolate Phytoconstituents of Barleria Noctiflora:*

- **Compound C1** was isolated from the *Barleria noctiflora* via column chromatography ethyl acetate extract. Below is a list of the chemical's spectrum and mechanical characteristics.
- **Homogeneity** was demonstrated by a specific location on TLC employing gel filtration G as an absorber and a solvent combination of varied polarity as developers. Table-25 shows the solvent system utilised and the accompanying Rf values.
- **Color reactions:** Compound C1 passed the Libermann-and Burchard's Salkowski tests, showing that it is a corticosteroid.
- **Compound C1 has a melting point of 170^{0}C.**

Table 5 Compound C1 TLC Profile

Phase of mobility	Ratio of solvents	The spot's Rf value
Methyl acetate: chloroform	70:30	0.53
Methanol: hexane	80:20	0.77
Benzene: chloroform	91:9	0.5

The following stretchings were all absorbed in the IR spectra shown in Figure-29: O-H stretching 3520-3420, C-H stretching aliphatic 2924, 2852, C-H stretching aromatic 1643, C=O stretching of flavones 1643, C=C aromatic stretching 1458, 1400, 1590, C-O stretching 1080, substituted aromatic, and finger print bands 597, 563.

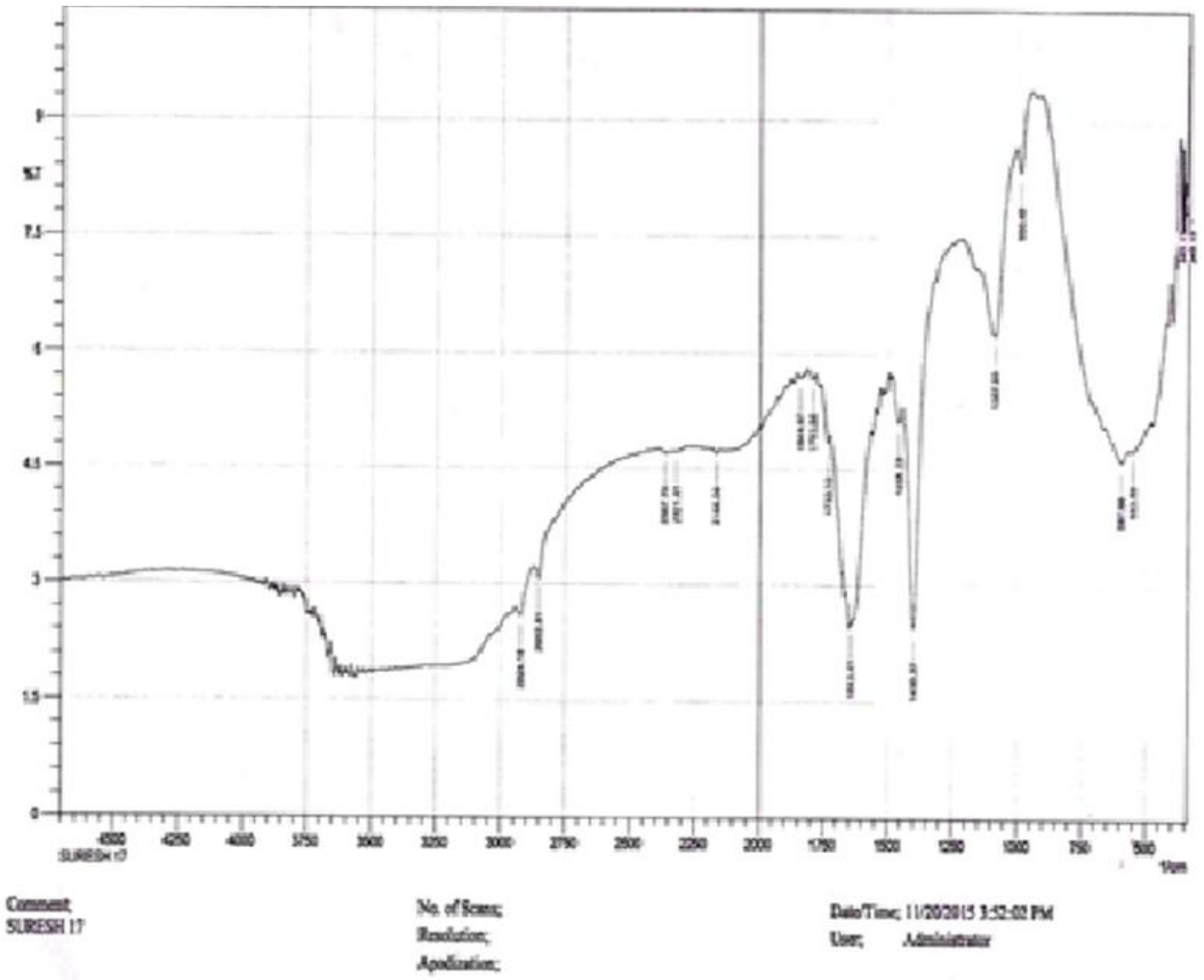

Fig 6 IR Spectra of Compound 1

VI. CONCLUSION

The current work offers the first detailed examination of the *Barleria noctiflora* plant from both a chemical and biological perspective. Antioxidant effects of plant extracts were shown *in vitro*. *Barleria noctiflora* ethanolic extract was shown to be the most effective. Studies on rats showed that the ethyl acetate portion of *Barleria noctiflora* has powerful hypoglycemia effects. The action was shown using biochemical and histological data. It was discovered that the *Barleria noctiflora* ethyl acetate fraction had in vivo wound healing activities in both the excision and incision models. Acyclic, unsaturated ketone molecule was identified by phytochemical study.

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