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Qualitative and Quantitative analysis, Antibacterial and Antioxidant Activity of Bulb in *Ledebouria revoluta* (*L.f.*) Jessop

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Abstract

This study was aimed to investigate the phytochemical constituents, antibacterial and antioxidant activity of *Ledebouria revoluta* bulb extract. Phytochemical analysis revealed the presence of active ingredients such as Alkaloids, saponins, carbohydrates, oils and resins. The antibacterial activity with three different species B.*subtilis, S.aureus, S.epidermis*. They are significant growth inhibition of human pathogens. *S.epidermis* species are have the highest inhibition growth in compare than other two species. The phytoconstituent of the *Ledebouria revoluta* bulb extract were analyzed by DPPH (IC_{50}=27.60µg/ml) and ABTS (IC_{50}=35.81µg/ml) TAC (IC_{50}=27.97µg/ml).

Key words: *Quantitative and Qualitative analysis, Antibacterial, Antioxidant and Ledebouria revoluta bulb extract.*

1. Introduction

Therapeutic plants are an incredible significance to the wellbeing of people and networks. The therapeutic estimations of these plants lies in some synthetic substances that deliver a clear physiological activity on the human body (Edeoga et al., 2005).The utilization of plants by man to treat normal illnesses is time immemorial and a large number of the conventional drugs are as yet included as a component of the constant treatment of different ailments (Henrich et al., 2002). About 60% of the all out worldwide populace stays reliant on conventional medications for their medicinal services framework (Kumar et al., 2004). In India a large number of animal varieties are known to have restorative qualities and the utilization of various parts of a few therapeutic plants to fix explicit afflictions has been in vogue since old occasions (Parekh et al., 2005).

As of late there has been an utilization of enthusiasm for the helpful capability of restorative plants as cancer prevention agents in diminishing such free radical-incited tissue damage (Pourmord et al., 2006). Extraction of bioactive mixes from restorative plants allows the showing of their physiological action. It likewise encourages the pharmacology ponders prompting union of an increasingly intense medication with diminishing poisonous quality (Manna and Abalka, 2000). The significance of plant auxiliary metabolites in prescription agribusiness and industry has prompted various examinations on the union, biosynthesis and organic action of these substances and much successful against different parasitic and bacterial pathogens. Greater part of these bioactive mixes are alkaloids, flavonoids, sterols, coumarins, quinines and monoterpenes. It is basic that ethnobotanical examines and phytochemical tests lead to some patentable and mechanically exploitable mixes for medication advancement (Jeruto Pascaline et al., 2011).

Countless having a place with a few concoction classes have been appeared to effectsly affect a wide range of microorganisms in vitro (Cowan, 1999). Plant items have been a piece of phytomedicines since time immemorial. This can be gotten from barks, leaves, blossoms, roots, organic products, seeds (Criagg et al., 2001). Learning of the compound constituents of plant is attractive on the grounds that such data will be an incentive for combination of complex concoction substances (Parech et al., 2005). Normally framing substances in higher plants have cancer prevention agent movement in the cells of the life form have be them (Larson, 1988, and Halliwell, 1997).The cancer prevention agent assume a vital job to ensure the human body against harm by responsive oxygen species. (Lollinger, 1981).Oxidative worry in diabetes exists together with a decrease in the cell reinforcement status (Collier et al.,1990).Supplementation of non-poisonous cancer prevention agents have a chemoprotective job in the

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diabetes (Logani and Davis, 1979). The cancer prevention agent is once in a while included with meat and poultry, which is keep the moderate oxidative debasement of fats. Which is viable on account of various components like free radical searching, chelating of professional oxidant metal particles arrangement. (Kershaw, 2000). The Ledebouria revoluta Jessop [Syn:Scillaindica (wight) pastry specialist or Scilla hyacinthina (Roth) J.F.Macbr and Drimiopsisbotryoids baker[syn: Drimiopsiskirkii baker] are bulbaceous types of the family Asperagaceae (The Plant List 2013).

2. Materials and Methods

2.1.Collection of Plant materials

The plants were gathered from Periyar University grounds, Salem. The investigation plant was related to the assistance standard greenery, for example, "Vegetation of administration of Tamil Nadu, Carnatic, India "(K.M. Mathew, 1988) and furthermore distinguished from the "Botanical Survey of Tamil Nadu".

2.2.Preparation of bulb extract

The new plant tests (globule) gathered were washed exclusively under faucet water and dried in room temperature for seven days. The plants were gathered and dried out little bits of shadow dry. The dried plant materials were granulate into powder. Around 50 gm of dry powdered plant material from each plant was removed by soxhlate mechanical assembly utilizing methanol dissolvable. The plant separates was then focused utilizing a revolving evaporator and the concentrated remaining concentrates were put away at 40 c in a dry impermeable compartment until further use.

2.3 Phytochemical Screening

Preliminary phytochemical analysis was carried out for methanol extracts of *Ledebouria revoluta* as per standard methods described by Brain and Turner 1975 and Evans 1996.

2.3.1. Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

2.3.2. Detection of Flavonoids

H2SO4 test: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

2.3.3. Detection of Phenols

Ferric chloride test: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

2.3.4. Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole sample was mixed with 2ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inner face was indicates the presence of terpenoids.

2.3.5. Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

2.3.6. Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

2.3.7. Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

2.3.8. Detection of Amino acid

Ninhydrin Test: take few drops of extracts and added with 0.25% ninhydrin reagent. Blue colour indicate the presence of amino acid.

2.3.9. Detection of Quanins

Sulphuric acid test: take few drops of extracts and added with sulphuric acid with form a red colour. Red colour indicates the presence of quanins.

2.3.10. Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

2.4. Quantitative Phytochemical analysis

2.4.1. Quantitative Estimation of Saponins

Test separate were broken down in 80% methanol, 2ml of Vanilin in ethanol was included, blended well and the 2ml of 72% sulphuric corrosive arrangement was included, blended well and warmed on a water shower at 600 c for 10min, absorbance was estimated at 544nm against reagent clear. Diosgenin is utilized as a standard material and

contrasted the test and Diosgenin reciprocals. (Diary of Pharmacognosy and Phytochemistry 2016; 5(2): 25-29)

2.4.1. Estimation of Carbohydrate

100 mg of test was hydrolysed in a bubbling cylinder with 5 ml of 2.5 N HCl in a bubbling water shower for a time of 3 hours. It was cooled at room temperature and strong sodium carbonate was included until the point that bubbling stops. The substance were centrifuged and the supernatant was made to 100 ml by utilizing refined water. From this 0.2 ml of test was pipetted out and made up the volume to one ml with refined water. At that point one ml of phenol reagent was included and pursued by 5.0 ml of sulphuric corrosive. The cylinders were kept at 25-30 C for 20 min. The absorbance was perused at 490 nm (Krishnaveni et al., 1984).

2.5. Antibacterial activity

The screening of methanol concentrates of various plant species for antibacterial movement was dictated by agar well dissemination technique (Perez et al., 1990; Parekh et al., 2005). The test microorganisms incorporate Gram's sure microbes are Bacillus subtilis, Staphyllococcus aureus, Staphyllococcus pneumonia. Culture Preparation: first Day : All testing society (GSTP1, GT132, HVT234) and pathogen (B.subtilus S. aureus S.pneumonia) was immunized in 5ml of Nutrient juices. At that point the cylinder was hatched in shaking hatchery at 37°C in 200rpm speed for 24 hrs. After brooding testing society (GSTP1, GT132, HVT234) soup was centrifuged at 10,000rpm for 20 mins and gathered culture supernatant utilized for further process. second Day: MHA medium was readied and cleaned. At that point the medium was poured in to the sterile petriplates. After medium hardening the pathogen was swapped on MHA plate for culture grass Well Diffusion Method : well was framed into the agar medium. 100µl of culture supernatant was stacked into the circle just as well plate additionally, at that point the plate was hatched at 37ºC for 24hrs. After hatching clear zone of restraint was seen on around the well.

2.6. Antioxidant Assay

2.6.1. DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging assay (Jain & Agrawal, 2008)

Procedure

Different concentrations (30 µl, 40µl & 50µl) of sample were taken and 50µl of 0.659mM DPPH dissolved in methanol solution was added make up to one with double distilled water. The tubes were incubated at 25 ºC for 20minutes. The absorbance value was recorded at 510 nm using shimadzu UV 1800 spectrophotometer. Above same procedure followed for control without sample.

2.6.2. ABTS radical scavenging Activity

Different concentrations (30 µl, 40µl & 50µl) of sample were taken and 0.3 ml of ABTS radical cation and 1.7 ml of phosphate buffer pH 7.4 was added. [ABTS solution: ABTS 2 mM (0.0548 gm in 50 ml) was prepared in distilled water. Potassium per sulphate 70 mM (0.0189g in 1ml) was prepared in distilled water. 200 µl of potassium persulphate and 50 ml of ABTS were mixed and used after 2 hrs]. The tubes were incubated at 25 ºC for 20minutes. The absorbance value was recorded at 734 nm using shimadzu UV 1800 spectrophotometer. Above same procedure followed for control without sample.

2.6.3. Total antioxidant capacity assay (Rajamanikandan et al 2011)

Different concentrations (30 µl, 40µl & 50µl) of extracts were taken and 1ml of reagent solution was added. [Reagent solution: 0.6M sulphuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate]. The tubes were capped and incubated in thermal block at 95ºC for 90 minutes. After the time interval the tubes were cool down at room temperature. The absorbance was recorded at 695 nm using shimadzu UV 1800 spectrophotometer.

Calculation of 50% Inhibitory Concentration (IC50)

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage inhibition (I %) was calculated using the formula,

$$I\ \% = \frac{(Ac - As)}{Ac} \times 100$$

Where Ac is the absorbance of the control and as is the absorbance of the sample.

3. Result and Discussion

3.1. Preliminary phytochemical analysis

3.1.1. Qualitative

The aftereffects of starter subjective phytochemical screening uncovered the nearness of Alkaloids, Saponins, sugar, oil and saps. The aftereffects of phytochemical examination are displayed in table.1. The Kubmarawa, 2007 and Mensah, 2008, completed the phytochemical screening of the different concentrates of Taraxacum officinale leaves were utilized steroids, tannins and triterpenoid and furthermore have different therapeutic qualities, for example, calming, against

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diabetic and pain relieving exercises and for focal sensory system movement. The significance of alkaloids, saponins and tannins in different anti-infection agents utilized in treating basic pathogenic strains has as of late detailed.

3.1.2 Quantitative

3.1.2. 1. The total saponin content of the methanol bulb extract

The all out saponin substance of the methanol concentrate of knob parts of Ledebouria revoluta was shifting broadly 3.306 mg/ml. (table.2). Shi, J., K. et al., 2004 uncovered that Traditionally the saponins have been broadly utilized as cleansers, as pesticides and molluscicides, notwithstanding their modern applications as frothing and surface dynamic specialists and furthermore have advantageous wellbeing impacts.

3.1.2.2. The total carbohydrate content of the methanol bulb extract

The total carbohydrate content of the methanol extract of bulb parts of *Ledebouria revoluta* was varying widely 3.291 mg/ml.(table.2).

3.2 Antimicrobial activities

The outcomes for the general screening for antibacterial action are appeared table 3. A complete three positive microbes species were explored. MIC estimations of dynamic concentrates are appeared table 3. Among the three species B.Subtilis demonstrated the most extreme inhibitory in knob of Ledebouria revoluta. Poor inhibitory movement was recognized against S.epidermis in the focus were 20 mg/ml. The high inhibitory esteem appeared in S. epidermidis in the convergence of 50mg/ml of globule concentrates of L.revoluta.(table.3, figure. 1, plate-2). Salari et al., 2006 uncovered that the eucalyptol demonstrated antibacterial action against some pathogenic microscopic organisms in the respiratory tract and due to its antimicrobial properties.it is additionally utilized in dental consideration and cleansers. Camila et al., 2013, did that the antibacterial capability of phthalate subordinates from plant, bis (2-ethylhexyl) extricated from streptomyces bangladheshiensis has been accounted for to demonstrate antibacterial movement against gram +ve microorganisms.

3.3 Antioxidant activity

3.3.1. DPPH radical scavenging activity

In the assessment of DPPH Antioxidant exercises, DPPH radical rummaging strategy was utilized to measure the hindrance of cell reinforcement. ABTS measure was utilized for the evaluation of cation ABTS radical hindrance. Productive fixation [IC50] was utilized to express cell reinforcement exercises. That are the measure of cell reinforcement expected to lessen the underlying radical fixation considerably. The least IC demonstrates better cancer prevention agent action. The globule remove restrain most elevated cell reinforcement exercises with the least IC50 values in these 52.95 ± 30 mg/ml. The knob separate show most minimal cell reinforcement exercises with the most noteworthy IC50 estimations of 77.18 ± 50mg/z. The DPPH radical rummaging exercises are (table 4, figure. 2). Singh et al., 2015, uncovered that DPPH is a steady free radical having a most extreme absorbance at 517nm in methanol and is rummaged from purple to yellow in the wake of tolerating an electron/proton radical to wind up a stable diamagnetic atom when cancer prevention agents are experienced.

3.3.2 ABTS radical scavenging activity

The globule extricate restrain most elevated cell reinforcement exercises with the least IC50 values in these 55.95+/ - 30 mg/ml. The knob remove show most minimal cell reinforcement exercises with the most elevated IC50 estimations of 68.71 +/ - 50mg/ml (table.5, figure.3). Re et al., 1999 uncovered that the ABTS test includes the oxidation of ABTS to shape a seriously green is exceptionally helpful for testing shaded nourishment extricates as ABTS free radical has greatest assimilation at 734nm as the majority of such concentrates don't ingest light at this wavelength.

3.3.3 Total antioxidant activity

The total IC indicates better antioxidant activity. The bulb extract inhibit highest antioxidant activities with the lowest IC_{50}values in these 55.95 ±30 mg/ml. The bulb extract exhibit lowest antioxidant activities with the highest IC_{50} values of 68.71± 50 mg/ml. (table.6, figure-4). Rubalakshmi and Karmegam, 2011, Siva praba et al., 2015, carried out total antioxidant activity is used for the analysis of fat-soluble and water- soluble antioxidants.

4 Conclusion

The present examination was completed by concentrate of *Ledebouria revoluta* globule of phytochemical, cancer prevention agent, antimicrobial action. The chose plant of Ledebouria revoluta has the wellspring of optional metabolites Alkaloids, Saponins, Carbohydrate, Oils and Resins. The Ledebouria revoluta was exceptionally wealthy in optional metabolites. Methanol concentrates of plant globule instigated high zone of restraint in staphylococcus epidermidis microbes. At long last Ledebouria revoluta as a potential possibility for antibacterial medication disclosure utilized cell reinforcement opposition. *Ledebouria revoluta* was completed for cancer prevention agent exercises. The

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Ledebouria revoluta globule extricate was demonstrated exceptionally restorative qualities.

Table: 1:Qualitative phytochemical analysis of L.R.B.(*Ledebouria revoluta* Bulb methanol extract)

Phytochemical	Observation	Extract
		L.R.B
Alkaloids Mayer's Test	Yellow cream precipitate	+
Flavonoids H_2SO_4	Orange colour precipitate	-
Phenols Ferric Chloride	Deep blue to Black colour formation	-
Terpenoids Salkowski's Test	Reddish Brown colour Precipitate	-
Saponin Forth Test	Bubbles are formed	+
Tannins Ferric Chloride Test	Dark Green Colour Precipitate	-
Carbohydrates Felling's Test	Green colour formed	+
Amino acid Ninhydrin Test	Blur colour formed	-
Quanins Sulphuric acid Test	Red colour fromed	-
Oils And Resins	Filter paper method	+

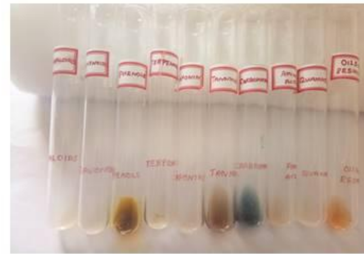

Table 2: Quantitative phytochemical Analysis of Methanol extracts of *Ledebouria revoluta* (Bulb) Sample Concentration (µg/ml)

S. No	Constituents	*Ledebouria revoluta* Bulb (µg/ml)
1	Saponin	3.306
2	Carbohydrate	3.291

Table: 3 Antibacterial activity of ***Ledebouria revoluta*** **(Bulb) Methanol** Extract against pathogenic Bacteria

S. No	**Sample Marking**	**Concentration**	**G+ve**		
			B.subtilis	***S. aureus***	***S. epidermidis***
1	***Ledebouria revoluta* (Bulb) Methanol** Extract	Positive Control Tetracycline	7mm	14mm	8mm
		Negative Control DMSO			NA
3		20µl	3mm	3mm	NA
4		30µl	4mm	4mm	3mm
5		40µl	5mm	5mm	6mm
6		50µl	6mm	6mm	7mm

NA - NO ACTIVITY

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Antioxidant Assay

Table-4 Free radical scavenging activities of *Ledebouria revoluta* (Bulb) determined by DPPH assay (%).

S.No	Sample marking and Compound	Radical scavenging activity at different concentrations (µg/mL)	% Inhibition of DPPH Antioxidant Activity	
			DPPH (µg/ml)	IC 50
1	*Ledebouria revoluta* Methanol Extract	30	52.95	
2		40	64.3	27.6
3		50	77.18	

Table-5 Free radical scavenging activities of Ledebouria revoluta (Bulb) determined by ABTS assay (%).

S.No	Sample marking and Compound	Radical scavenging activity at different concentrations (µg/mL)	% Inhibition of ABTS Antioxidant Activity	
			ABTS (µg/ml)	IC50
1	*Ledebouria revoluta* Methanol Extract	30	71.63	
2		40	76.73	35.81
3		50	81.42	

Table-6 Free radical scavenging activities of Ledebouria revoluta (Bulb) determined by TAC assay (%).

S.No	Sample marking and Compound	Radical scavenging activity at different concentrations (µg/mL)	% Inhibition TAC Antioxidant Activity	
			TAC (µg/ml)	IC50
1	*Ledebouria revoluta* Methanol Extract	30	55.95	27.97
2		40	65.9	
3		50	68.71	

Figure: 1 Antibacterial Activity

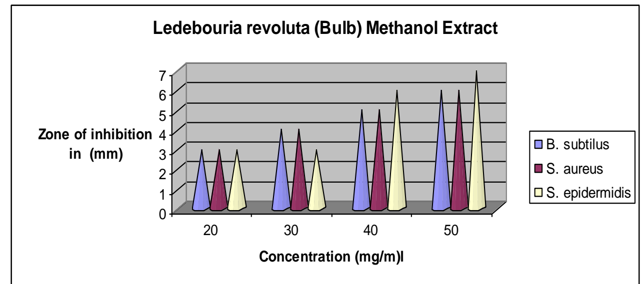


Figure-2 DPPH Scavenging activity of Methanol ectracts from *Ledebouria revoluta* (Bulb)

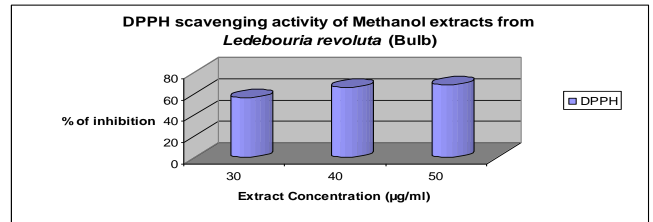


Figure-3 ABTS scavenging activity of Methanol extract from *Ledebouria revoluta* (Bulb)

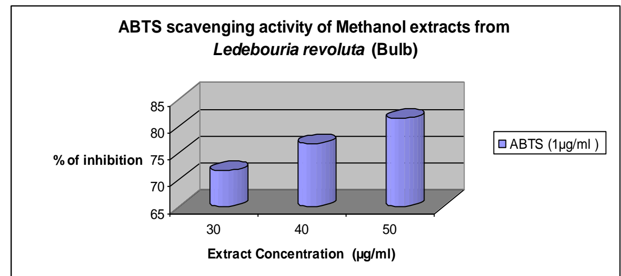


Figure-4 TAC scavenging activity of Methanol extract from *Ledebouria revoluta* (Bulb)

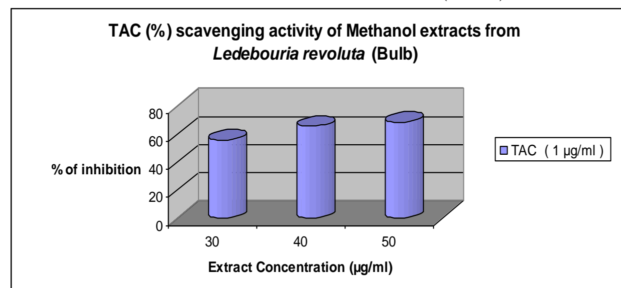
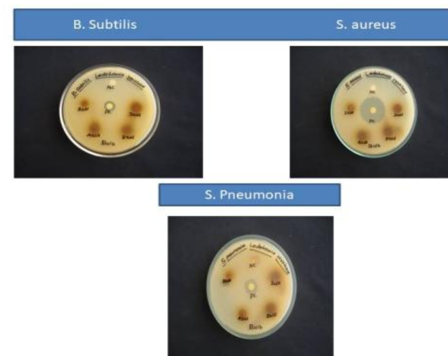


Plate: 1 Antibacterial Activity



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