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Phytochemical Composition and *in vitro* Antimicrobial, Antioxidant Activities of Ethanolic Extract of *Leptadenia reticulata* [W&A] Leaves

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Abstract: Objective: To investigate the phytochemical composition, *in vitro* antimicrobial and antioxidant properties of ethanolic extracts of *Leptadenia reticulata* [W&A] leaves. Methods: Ethanolic extracts were prepared from fresh dried leaves of *Leptadenia reticulata* [W&A] by hot continuous percolation method in Soxhlet apparatus. Ethanolic extract of *Leptadenia reticulata* [W&A] were subjected to identify phytochemical compounds by using GC-MS analysis. Ethanolic extract of *Leptadenia reticulata* [W&A] were tested for antimicrobial efficacy against Gram positive, Gram negative bacteria and fungi. The antimicrobial effect produced by ethanolic extract of *Leptadenia reticulata* [W&A] was comparable to that of ciprofloxacin and amphotericin which were used as standard. The antioxidant activity of ethanolic extracts of *Leptadenia reticulata* [W&A] estimated by total reduction capability, superoxide anion scavenging activity, free radical scavenging activity, hydrogen peroxide scavenging activity were determined treating with different concentrations of Vitamin C and Butylated hydroxyl anisole (BHA) as standard antioxidant compound. Results: A number of 10 phytoconstituents were identified by GC-MS. Ethanolic extract of *Leptadenia reticulata* [W&A] exhibits antimicrobial activity same as that compared to standard drugs ciprofloxacin and amphotericin. The extract possesses a significant antioxidant potential compared to that of the standards Butylated hydroxyl anisole and L-ascorbic acid. Conclusion: These results concluded that *Leptadenia reticulata* [W&A] leaves possess high antimicrobial and antioxidant activity and can be used for the development of a safe herbal antioxidant and antimicrobial agents.

Key words: Leptadenia Reticulata [W&A] · GC-MS · Antimicrobial Activity · Antioxidant Activity

INTRODUCTION

Plants, owing to its medicinal value have continued to play a dominant role in the maintenance of human health since ancient times. The world health organization estimates that the plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [1]. Turkish people have a tradition of using a number of plant species for the treatment of infectious diseases and various ailments [2]. Traditional and folkloric medicines play important role in health services around the world. About three quarter of the world's population rely on plant and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. The subcontinent is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate, which have a bearing on its vegetation and floristic composition. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties [3]. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. *Leptadenia reticulata* [W&A] belongs to the family Asclepiadaceae and is commonly known as Jiwanti. It grows in the sub-Himalayan tracts of Punjab, Gujrat, Uttar Pradesh and throughout peninsular India, ascending to an altitude of 900 m. According to Ayurveda, jiwanti is a jeevana tonic that boosts the energy level of the body. It is mainly recommended to those who suffer from weak debility or a lack of energy. Jiwanti has been claimed to be useful as galactagogue, antibacterial, lactogenic,

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hypotensive, restorative, tonic and hypoglycaemic activity [4]. From the above information the present investigation was undertaken which deals with the studies of the ethanolic extracts of *Leptadenia reticulata* [W&A] against various Gram positive, Gram negative bacteria and fungi, the result of which are reported in the present communication. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [5]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [6]. GC-MS is a method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. In the present paper, examined the chemical composition of the ethanol extract of *Leptadenia reticulata* [W&A] using GC-MS. In addition, the antimicrobial and antioxidant activities of the ethanolic extract of *Leptadenia reticulata* [W&A] were also investigated.

MATERIALS AND METHODS

Preparation of Plant Extract: Leptadenia reticulata [W&A] leaves were collected from the forest of kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai and authenticated by Botanist Chelladurai with the voucher specimen No (CCRAS-168/2011). Voucher specimen has been prepared and preserved in the Department of Pharmacy, Annamalai University for the future reference. Fresh plant leaves were shade dried at room temperature, ground into fine powder and stored in airtight containers. Then extracted (amount 500 g) with solvents of increasing polarity such as petroleum ether, ethyl acetate and ethanol, for 72 hours with each solvent, by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis: GC-MS analysis of the ethanolic extract of *Leptadenia reticulata* [W&A] was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% biphenyl/95% dimethyl poly siloxane) fused with a capillary column (30 × 0.25 ìm × 0.25 ìm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 ìl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectrum was taken at 70 eV; a scan interval of 0.5 sec and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass version-5.2.

Identification of Phytoconstituents: Interpretation of the GC-MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Microorganisms: The following microbial strains were obtained from National Chemical Laboratory, Pune, India and used to the study antibacterial activity of ethanolic extract of *Leptadenia reticulata* [W&A]. Microorganisms for antibacterial activity (Gram positive organisms) *Bacillus Subtilis* (ATCC-6633), *Staphylococcus aereus* (ATCC-29737) and (Gram negative organisms) *Escherichia coli* (ATCC-8739), *Pseudomonas aeruginosa* (ATCC-25619) and *Klebsiella pneumonia* (ATCC-12228) and (fungi) *Aspergillus flavus* (ATCC-2535), *Aspergillus niger* (ATCC-3590).

Evaluation of Antimicrobial Activity

Paper Disc Diffusion Method: Plant extracts were diluted at a concentration of 25, 50, 100, 200mg/ml in 5% aqueous DMSO. The antimicrobial assay was performed by disc diffusion method [7]. Agar plates were prepared and the

test microorganisms were inoculated by the spread plate method. Filter paper discs approximately 6mm in diameter were soaked with 15ìl of the leaf extract and placed in the previously prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, centre to centre. The agar plates were then incubated at 37°C. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the ciprofloxacin and amphotericin was used as control. The experiment was performed in triplicate and the mean values of results were given in Table-2.

In Vitro Antioxidant Assay

Total Reduction Capability: The reduction capacity of a compound indicates its antioxidant potential. The transformation of Fe3+ to Fe2+ was investigated for measuring the reductive ability. Total reduction capability of ethanolic extract of *Leptadenia reticulata* [W&A] was estimated [8]. Various concentrations of *Leptadenia reticulata* [W&A] (25-75µg.ml-1) were prepared in 2.5 ml of 0.2M phosphate buffer pH 6.6. To every concentration, 2.5 ml of 1% potassium ferricyanide [K3Fe-(CN) 6] and 2.5 ml of 10% trichloroacetic acid were added. The mixture was incubated at 50° C for 20 min and centrifuged at 2000 rpm for 30 minutes. Supernatant solution (2.5ml) was mixed with equal volume of distilled water and 0.5ml of 0.1% FeCl3. The absorbance of the solution was measured at 700 nm using spectrophotometer (Shimadzu 160 UVPC). Higher absorbance was the indication of greater reduction power and it was compared with the absorbance capacity of standard antioxidants, ascorbic acid and butylated hydroxyl anisole (BHA).

Superoxide Anion Activity: Superoxide anion is an oxygen-centred radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalent reduce molecular oxygen [9]. Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleodide (PMS-NADH) and oxygen. It was assayed by the reduction of nitro blue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris-Hcl buffer (100mM, pH 7.4) containing 0.75 ml of NBT (300µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of various concentration of extracts of *Leptadenia reticulata* [W&A] such as 25, 50, 75, 100, 250 and 500 µg.ml-1. The reaction begins with the addition of 0.75 ml of PMS (120 µM) to the mixture. After 5 minute of incubation at room temperature, the absorbance at 560nm was measured using a spectrophotometer (Shimadzu 160 UVPC). The percentage of super oxide anion scavenging activity was calculated using the following equation. The percentage scavenging of superoxide anion radical = [(A0-A1)/A0×100], where A0 is the absorbance of the control reaction (blank, without extract) and A1 is the absorbance in presence of the standard or test.

Free Radical Scavenging Activity: DPPH is used as a free radical to evaluate antioxidant activity of natural compounds. The degree of its discoloration is attributed to the hydrogen donating ability to test compound. The free radical scavenging activity of *Leptadenia reticulata* [W&A] was estimated by using 1, 1- diphenyl – picryl – hydrazil (DPPH) [10]. In this experiment 0.1mM solution of DPPH in ethanol was prepared. 1ml of the DPPH solution was added to 3 ml of various concentrations of *Leptadenia reticulata* [W&A] such as 25, 50, 75, 100,250 and 500 µg.ml-1. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance was measured at 517 nm using UV-Visible spectrophotometer (Shimadzu 160 UVPC). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of DPPH scavenging effect was calculated.

Hydrogen Peroxide Activity: The ability of the extracts of *Leptadenia reticulata* [W&A] to scavenge hydrogen peroxide was determined [11]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Various concentrations of *Leptadenia reticulata* [W&A] 25, 50, 75, 100, 250, 500 µg.ml-1 in distilled water was added to hydrogen peroxide solution (0.6ml, 40mM). After 19 minute the absorbance of hydrogen peroxide was measured at 230 nm against a blank solution consisting of phosphate buffer without hydrogen peroxide. The concentration was determined spectrophotometric at 230 nm (Shimadzu 160 UVPC). The percentage of scavenging activity of hydrogen peroxide was calculated.

Statistical Analysis: All experiments were done in triplicate and results were reported as mean ± SEM. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests. $*P < 0.05$.

RESULTS AND DISCUSSION

Identification of Compounds: The retention time, name of the compound, molecular weight and percentage peak area of the components of the test materials were ascertained and presented in Table-1 and GC-MS chromatogram illustrated in Figure-1.

Antimicrobial Activities: Ethanolic extract of *Leptadenia reticulata* [W&A] exhibits a good antimicrobial activity against all the microorganisms tested, which was summarized in Table-2. The zone of inhibition exhibited by ethanol extract was compared with standard antibiotics. This study showed that ethanolic extract of *Leptadenia reticulata* [W&A] exhibits both antifungal and as well as antibacterial activity. The active principles in ethanolic extract of *Leptadenia reticulata* [W&A] identified by GC-MS were clearly shown in Table-1. The compounds to have antimicrobial property were monoterpenes, sesquiterpenes, aromatic aldehydes and ketones [12]. *Leptadenia reticulata* [W&A] leaves were rich in essential oil containing monoterpenes, triterpenes and sesquiterpenes which are responsible for its antimicrobial property. It was reported that 60% of essential oil derivatives examined were inhibiting fungi,

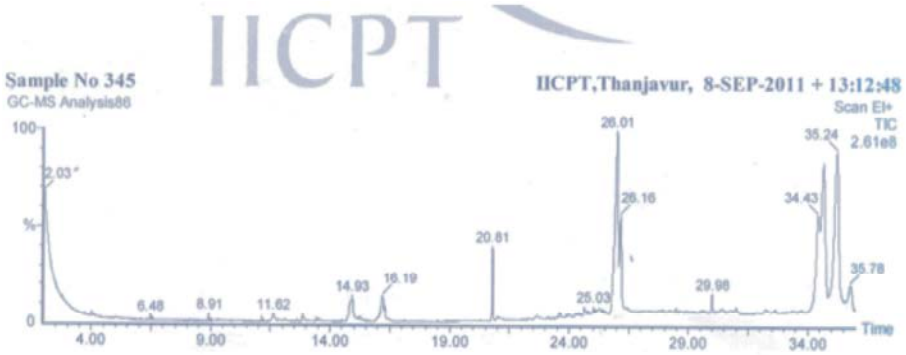

Fig 1: GC-MS Chromatogram of ethanolic extract of *Leptadenia reticulata* [W&A]

Table 1: Components identified in the ethanolic extract of *Leptadenia reticulata* [W&A]

No	RT	Name of the compound	Molecular Formula	MW	Peak area %
1	6.48	1-Dodecene	$C_{12}H_{24}$	168	0.30
2	8.91	3-Tetradecene,(Z)-	$C_{14}H_{28}$	196	0.31
3	11.62	Methyl-10-undecenoate	$C_{20}H_{40}O$	296	1.21
4	12.85	Cyclopentaneundecanoicacid,Methyl ester	$C_{17}H_{32}O_2$	268	1.08
5	14.93	Urs-12-en-24-oic acid,3-oxo-.methyl ester(+)-	$C_{31}H_{48}O_3$	468	8.69
6	16.19	Azulene.1,2,3,4,5,6,7,8,8á-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,(1s-(1a,7a,8aa)-	$C_{15}H_{24}$	204	7.34
7	20.81	Quercetin	$C_{15}H_{10}O_7$	390	5.18
8	26.01	Phytol	$C_{20}H_{40}O$	296	47.68
9	29.98	Lupeol	$C_{30}H_{50}O$	426	2.00
10	34.43	Isopropyl Linoleate	$C_{12}H_{38}O_2$	322	26.22

Table 2: Antimicrobial activity of ethanol extract of *Leptadenia reticulata* [W&A]

	Diameter of zones of inhibition in mm					
	Ethanolic extracts in mg/ml conc.				Ciprofloxacin	Amphotericin
Micro organisms	200	100	50	25	(10mcg/disc)	(10mcg/disc)
Bacillus Subtilis	4+	2+	1+	-	26	-
Staphylococcus aereus	3+	2+	1+	1+	24	-
Escherichia coli	4+	3+	2+	1+	25	-
Pseudomonas aeruginosa	4+	3+	2+	-	28	-
Klebsiella Pneumoniae	4+	3+	2+	-	24	-
Aspergillus flavus	3+	2+	1+	1+	-	20
Aspergillus niger	3+	2+	1+	1+	-	20

1+ indicates zone of inhibition in average of 7 to 10 mm; 2+ indicates zone of inhibition in average of 11 to 14 mm; 3+ indicates zone of inhibition in average of 15 to 18 mm; 4+ indicates zone of inhibition in average of 19 to 22 mm; - No activity

Table 3: Effect of Ethanolic Extract of DV and LR in Total Reduction Capability

	Absorbance		
Concentration (µg/ml)	l-Ascorbic acid	BHA	ETLR
25	0.931±0.014*	1.63±0.051*	1.093±0.019*
50	1.431±0.002*	2.07±0.034*	1.698±0.002*
75	2.040±0.002*	2.84±0.042*	2.143±0.008*

n=3. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests. *$P < 0.05$

while 30% inhibiting bacteria [13]. The mechanisms behind the antibacterial activity are cytoplasm granulation, cytoplasmic membrane rupture and inactivation or inhibition of intracellular enzymes. When coming to antifungal activity, lytic enzymes act on the fungal cell wall, causing breakage of β-1,3glycan, β-1,6glycan and chitin polymers [14]. Terpenes are phenolic compounds that exhibit the antimicrobial activity and mostly mono and sesquiterpenes are active against bacteria, fungi, virus and protozoa [15]. The terpenes identified in ethanolic extract of *Leptadenia reticulata* [W&A] of Phytol and Lupeol exhibits antimicrobial activity. Terpenes are found in latex and resins of some plants and physiological function of these compounds is generally believed to be a chemical defence against certain pathogens causing human and animal disease [16]. The activity is a function of the lipophilic properties of the constituent terpenes, the potency of their functional groups and their aqueous solubility [17]. Summarizing these results, we conclude that the antimicrobial activity of the *Leptadenia reticulata* [W&A] was mainly due to presence of phenolic compounds monoterpenes, triterpenes and sesquiterpenes.

Effect on Total Reduction Capability: The effect of ethanolic extract of *Leptadenia reticulata* [W&A] (ETLR) with the concentrations of 25, 50 and 75µg.ml-1 were tested for total reduction capability. From the study it was observed that ETLR showed significant (p<0.05) reduction potential and the results are comparable to that of standard antioxidants such as l-ascorbic acid and BHA. The order of total reduction capability of ETLR and the standard antioxidants are BHA> ETLR> l-ascorbic acid. The results were shown in table-3.The reduction property of ETLR increased proportionately to its concentrations. ETLR showed higher antioxidant effect than l-ascorbic acid and lower than BHA at all the concentrations. The component responsible for the antioxidant activity is quercetin [18]. The Fe3+ to Fe2+ transformation in the presence of ETLR was investigated and found to be significantly reducing. The reducing capacity of a compound may serve as significant indicator of its potential antioxidant activity. The antioxidant activity of a compound has been attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [19].

Effect on Superoxide Anion Radical Scavenging Activity: The effect of ETLR with the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were tested for their superoxide anion radical scavenging activity. The percentage of inhibition of superoxide radical generation of ETLR at the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were determined and the results were compared with the same concentrations of standard antioxidants such as l-ascorbic acid and BHA. From the study it has been observed that the ETLR has showed a significant (p<0.05) activity when compared to the standards. The percentage of inhibition of superoxide generation by ETLR at 500µg.ml-1 concentration was found to be 74.67%, whereas, l-ascorbic acid and BHA were found to be 78.82% and 88.68%, respectively. The orders of superoxide radical scavenging activity of petroleum ether extract and standard antioxidants are BHA> l-ascorbic acid> ETLR. ETLR showed almost equivalent antioxidant activity as that of the standards. The results were shown in Table- 4. Superoxide anion radicals are produced endogenously by flavoenzymes like xanthine oxidase, which convert hypoxanthine to xanthine and subsequently to uric acid in ischemia. In the phenazine methosulfate-nicotinamide adenine dinucleodide (PMS-NADH) system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The reduction in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reactive mixture. Superoxide anions generation found to be reduced in the presence of ETLR in hypoxanthine/ xanthine oxidase reaction. In this system there are two possibilities: either the plant extracts scavenge the O2 or they inhibit the xanthine oxidase activity.

Table 4: Effect of Ethanolic Extract of DV and LR in Superoxide Anion Radical Scavenging Activity

	Percentage Inhibition of Superoxide Generation		
Concentration (µg/ml)	l-Ascorbic acid	BHA	ETLR
25	10.231±1.214*	15.632±1.251*	0.845±0.022
50	15.831±0.682*	19.873±0.630*	3.153±0.043
75	18.240±0.002*	26.843±1.242*	16.452±0.786*
100	28.478±0.249*	52.584±1.870*	26.985±0.279*
250	70.158±2.297*	82.232±0.271*	60.857±0.326*
500	78.835±0.289*	88.693±1.412*	74.675±0.421*

n=3. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests. *$P < 0.05$

Table 5: Effect of Ethanolic Extract of DV and LR in Free Radical Scavenging Activity

	Percentage Inhibition of Free Radical Generation		
Concentration (µg/ml)	l-Ascorbic acid	BHA	ETLR
25	51.710±0.406*	43.632±1.551*	28.438±1.198
50	55.831±0.482*	57.573±0.330*	27.546±0.563
75	57.740±1.572*	60.643±1.242*	30.254±0.862
100	59.878±1.749*	66.254±1.870*	48.658±1.654*
250	66.458±1.797*	69.732±0.271*	64.546±0.243*
500	68.235±1.289*	71.243±1.282*	66.765±1.345*

n=3. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests. *$P < 0.05$.

Table 6: Effect of Ethanolic Extract of DV and LR in Hydrogen Peroxide Scavenging Activity

	Percentage Inhibition of Hydrogen Peroxide Generation		
Concentration (µg/ml)	l-Ascorbic acid	BHA	ETLR
25	13.720±0.046*	15.232±2.251*	10.487±1.785*
50	24.431±0.682*	18.573±0.480*	23.563±0.675*
75	35.940±0.172*	37.643±0.342*	34.547±0.985*
100	49.178±0.189*	48.654±0.270*	45.365±0.769*
250	56.458±0.797*	58.742±0.771*	55.859±1.467*
500	58.835±0.389*	67.643±0.282*	57.086±1.375*

n=3. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests. *$P < 0.05$

Effect on Free Radical Scavenging Activity: The effect of ETLR with the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were tested for their free radical scavenging activity. The percentage of inhibition of superoxide radical generation of ETLR at the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were determined and the results were compared with the same concentrations of to that of standard antioxidants such as l-ascorbic acid and BHA. From the study it has been observed that the ETLR at 500µg.ml-1 significantly reduced the concentration of DPPH radical formation and the results were compared to the standards. The orders of free radical scavenging activity of ETLR and standard antioxidants at a concentration of 500µg.ml-1 were; BHA > l-ascorbic acid > ETLR and the scavenging activity in percentage were 71.24, 68.23, 66.76 respectively. Free radical scavenging activity of ETLR increased proportionately and the results were shown in Table-5. The model of scavenging the stable DPPH radical is the widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. The effect of antioxidants on DPPH radical scavenging is supposed due to their hydrogen donating ability. DPPH is a stable free radical and accepts hydrogen radical to become a stable free radical and accepts an electron on hydrogen radical to become a stable diamagnetic molecule [20]. The decrease in absorbance of DPPH radical caused by antioxidants, due to the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. These results revealed that the ETLR is a free radical inhibitor.

Effect on Hydrogen Peroxide Scavenging Activity: The effect of ETLR with the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were tested for their hydrogen

peroxide radical scavenging activity. The percentage of inhibition of hydrogen peroxide radical scavenging of ETLR at the concentrations of 25, 50, 75, 100, 250 and 500µg.ml-1 were determined and the results were compared with the same concentrations of that of standard antioxidants such as l-ascorbic acid and BHA. From the study it has been observed that ETLR showed a significant (p<0.05) promising activity at all concentrations and the result were similar to the standards. The percentage of hydrogen peroxide scavenging activity of l-ascorbic acid and BHA and ETLR at a concentration of 500µg.ml-1 was found to be 58.84, 67.64 and 57.08 respectively. The order of hydrogen peroxide scavenging activity of ETLR and standard antioxidants were; BHA> ETLR> l-ascorbic acid. The results were shown in Table-6. H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. These results revealed that the ETLR have hydrogen peroxide scavenging activity. The crude extract itself shows comparably high antioxidant potential than standards. If the compound is purified it can be a better alternative and potential antioxidant for medicinal purposes.

CONCLUSION

To conclude the study, the extract has demonstrated significant antimicrobial and *in-vitro* antioxidant activity, when compared with standard drugs. This study scientifically supports the usage of leaves of *Leptadenia reticulata* [W&A] as a remedy for various superficial antimicrobial infections and antioxidants as a traditional medicine. We hope that the study emphasizes the accuracy and efficacy of traditional remedies and that it inspires people to realize the importance of protecting natural resources for sustainable use for its potent pharmaceuticals.

Conflict of Interest: These authors have no conflict of interest to declare.

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