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# Phytochemical evaluation of arial part of *Leptadenia reticulata (Retz)* for poly phenolic compound and free scavenging activity.

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**Abstract**
Phytochemical evaluation of arial part of *Leptadenia reticulata (Retz)* for poly phenolic compound and free scavenging activity. Thus to determination the Total Phenolic Content (TPC) quantified by Folin–Ciocalteu method and free radical scavenging (antioxidant) activity by in LRUSM & Acetone Extract (LRA) & methanolic (LRM) and aqueous (LRAQ) extracts of Arial part of the *Leptadenia reticulata (Retz),*. Currently, there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine in number of degenerative diseases.Total Phenolic content was found to be 2.77 % w/w in LRM & 1.33 % w/w in LRA eq Gallic acid and total flavanoid content was found to be 3.21% w/w eq. to quarcetin in LRM. (IC50) was calculated from the graph plotting inhibition percentage against extract concentration .indicated that the extracts (LRM, LRA & LRUSM) of *L.Reticulata* have notably reduced the stable free radical of DPPH (Graph 2, 3, 4 and5) to the yellow-colored Diphenyl picryl hydrazyl with an IC50 values 56.66, 55.55 and 47.20 µg/ml respectively showed in (Table 1) comparison with Ascorbic acid (Vitamin-C) (IC50 = 40.70 µg/ml) And BHA( IC50 = 61.74 µg/ml). Free radical activity of various extract of *Leptadenia reticulata* is due to present of polyphenolic compound present in LRUSM and LRM.

**Key Words**
*Leptadenia reticulata (Retz),* Total Phenol Content (TPC), free radical scavenging.

## Introduction

Herb is concentrated food that provides nutritional value like vitamins, minerals along with health benefits to the human body. They are used by man since the beginning of civilization. Today health-conscious public is now realizing that herbs, in combined with proper diet and exercise program can help them to achieve and maintain good health. Ayurveda that improved the general health of body by scavenging free radical. Rasayana is one of the classes of ayurveda that improved the general health of the body.

It nourishes and rejuvenates the body and increases Memory, longevity, immunomodulation and adoption. *Leptadenia reticulata (Retz)* or jivanti is a much branched twining shrub belongs to Asclepiadaceae family.

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Jivanti grows throughout India, flower are greenish yellow, in many flowered cymes or subaxillary cymes, the follicles are sub woody and turgid Stem is cylindrical and bent occasionally at places. It is 5 to 10 cm long, 0.5 to 2.5 mm in diameter. The surface is rough, longitudinally ridged, Wrinkled and furrowed, transversely cracked and with vertically elongated lenticels at places. Externally whites brown, internally pale brown, fracture short and splintery, odour and taste are sweet. The bark is yellowish brown, corky, deeply cracked. Leaves are ovate to cordate, 4 to 8 cm long, 2 to 5.5 cm broad, entire, acute, subacute, to mucronate, base symmetrical, petiole 1 to 3 cm long, pubescent below, green colour, and taste and odour are characteristic. the root are externally rough, white with longitudinal ridges and furrows and in transverse section the wide cork, lignified stone celllayer, madullary rays can be seen. The root size varies from 3 to 10 cm in length and 1.5 to 5 mm in diameter. Flowering stage occurs in May and June, while fruiting begin in October and continues up to November [1, 2]. Jivanti is sweet in taste and useful in alleviating all the three doshas, namely, vata, pitta and kapha. Mainly the roots and the whole plant are used for medicinal purposes so it also included vitalizing group [3]. They represent a potential source of new compounds with antioxidant activity. Antioxidants help organisms deal with oxidative stress, caused byfree radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stabilityc[4]. Extensive review on the effect of free radicals and antioxidants in normal physiological functions and human were studied[5]. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[6]. A Tannin, flavonoids, steroids, terpenoids and saponins have been isolated and characterized from genus *Leptadenia*[15,16.] Previous phytochemical investigations of showed the presence of flavonoids, two sesquiterpene lactones, gallic acid, β-sitosterol, geranial [7]. The analysis of phenolic compounds in plants is of considerable commercial importance, since it is known that they contribute to the flavor and antioxidant property [9]. However, no attention has been paid to its comparative Total Phenolic Content (TPC) and antioxidant status of stem extracts. Thus a common spectrophotometric method for total poly phenol content according to Follin-Ciocalteu has been widely used in the area of oncology and viticulture[19]. In-vitro antioxidant property by common DPPH Scavenging method. In the present investigation these *in-vitro* studies were carried out for the first time to study the comparative properties of total polyphenol content [10].

**Material and method**

**Plant material**

The plant material of *Leptadenia reticulata (Retz.)* was collected from

Late Miss misha medicinal plant garden Dhanvantary pharmacy college, Kim Surat, Gujarat. All the collected material was authenticated at the agricultural university voucher specimen was deposited in herbarium, (Voucher specimen no. 37) of for future reference.

**Preparation of extracts**

The freshly collected arial were washed; shade dried and was treated with a mechanical pulverizer for size reduction. The fine powder was collected and was used for preparation of extracts. The powder was successively extracted with pet ether (60-80), acetone using soxhlet apparatus for 72 h. Finally, after removal of acetone, marc of *Leptadenia reticulata* was subjected to cold maceration with 70 % methanol. All extracts were concentrated separately under reduced pressure. Finaly saponification of petether extract to get USM. The percentage yields of extracts were 2.64 % w/w (PLR) from 32.7% ULR, 8.01%w/w (ALR) and 12.11% w/w (MLR). The extracts were kept in desiccators for further use [16, 17, 18].

**Chemicals and instruments**

REAGENTS: 2, 2-diphenyl-1-picryl hydrazy l (DPPH), Ascorbic acid, Folin Ciocatteu's (FC) phenolreagent, Gallic acid, anhydrous sodium carbonate, allother reagents used were of analytical grade. UVspectra were recorded in Perkin-Elmer model Lambda-25, UV-Visible spectrophotometer.

**Determination of total phenolic content**

The content of Total Phenolic in Arial part of *Leptadenia reticulate* plant extarct (LRM, LRA) extracts were determined spectrophotometrically using Folin–Ciocalteu reagent by the method of Macdonald et. al with modifications[11]. Calibration curve was prepared by mixing ethanolic solution of gallic acid (2 to 10 µg/ml) with 5ml Folin-Ciocalteu reagent(diluted tenfold) and Sodium carbonate solution in Distilled Water (4ml, 0.7 M). The absorption was measured at 765 nm using a UV-Vis Perkin-Elmer spectrophotometer model Lambda-25. One millimetre of plant extracts (10mg/10m- 0.1 -10 ml= 100ug/ml ) was mixed instead of 1ml gallic acid with the same reagents as described above in three different Test tubes and after 1 hour the absorption was measured to determination the total phenolic contents. The absorbance was measured against a reagent blank, which was composed of the same reagents except test extract. The Gallic acid standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) ($y = 0.0748x - 0.0778$, $R^2 = 0.998$), where y is absorbance and x is concentration. (Fig.1) Total content of phenolic in the plant extracts were expressed as gallic acid equivalents and were calculated by the formula: T = C X V/M Where, T=total content of phenolic compounds, Milligram per gram plant extract, in GAE; C=the concentration of gallic acid established from the calibration curve, milligram per milliliter; V=the volume of extract, millilitre; M=the weight of Result

**Evaluation of antioxidant activity**

This method was given by Brand-Williams, Cuvelier, and Berset (1995)[12] and later was modified by Sanchez-Moreno, Larrauri, and Saura-Calixto (1998)[13]. It is one of the most extensively used antioxidant assay for plant samples. This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity of the extracts (AIM, AIHA & AIA) were examined *in vitro* using DPPH radical [14]. The radical scavenging activities of the plant extracts against DPPH radical (Sigma Aldrich) were determined by UV Perkin Elmer UV-Vis model Lambda 25 spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by various scientists[11,14]. Hydrogen atom or electron-donating ability of the stem extracts was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable DPPH radical as a reagent [15]. One ml of various concentrations of the (LRM 20 - 100 µg/ml, 20 - 100 µg/ml samples in methanol and LRUSM 20 - 100 µg/ml sample re dissolved in 5% ethanol) extracts were added to 3ml of methanol followed by 0.5 ml of1mM methanolic solution of DPPH. After incubation period at room temperature, the absorbance was reading against a blank (A blank solution was prepared containing the same amount of methanol and DPPH except the test compound). Ascorbic acid (Vitamins-C) was used as the antioxidant standard at concentrations of 0.005 - 0.06 mg/ml. The radical scavenging activity (Inhibition of DPPH free radical inpercent) was calculated using the following formula,

$$\% \text{ Inhibition} = \frac{[Ab - Aa]}{Ab \times 100}$$

Where,

Ab- is the absorption of the blank sample (containing all reagents except the test compound)

Aa- is the absorption of the test compound.

Sample concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentage against extract concentration .indicated that the extracts (LRM, LRA & LRUSM) of *L.Reticulata* have notably reduced the stable free radical of DPPH (Graph 2, 3, 4 and5) to the yellow-colored Diphenyl picryl hydrazyl with an IC50 values (theconcentration that Inhibits 50% of the DPPH radical) 56.66, 55.55 and 47.20 µg/ml respectively showed in (Table 1) comparison with Ascorbic acid (Vitamin-C) (IC50 = 40.70 µg/ml) And BHA ( IC50 = 61.74 µg/ml).

**Results**

**Total phenolic content**

The total phenolic content of the *Leptadenia reticulata* extracts (LRM, LRA) measured by Folin Cicalteu reagents were 1.33 mg %, 2.77 mg % and. respectively in terms of gallic acid equivalent (GAE)showed in Table No. 1,2.

**Antioxidant activity (DPPH assay)**

Radical Scavenging Increased with Antioxidant. The results of scavenging effect of tested plant extracts on DPPH radical are given in (Table NO.3). These results indicated that the extracts (LRM, LRA & LRUSM ) of arial part of *L.Reticulata* have notably reduced the stable free radical of DPPH (Graph 2, 3, 4 and 5) to the yellow-colored Diphenyl picryl hydrazyl with an IC50 values (the concentration that Inhibits 50% of the DPPH radical) with an IC50 values 56.66 µg/ml, 55.55 µg/ml and 47.20 µg /ml respectively showed in (Table no. 3) result well comparison with Ascorbic acid (Vitamin-C) (IC50 = 40.70 µg/ml) And BHA( IC50 = 61.74 µg /ml).

**Discussion**

The TPC of the *L. reticulata* extract were found more in LRA than LRM. The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron became paired off in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichio metrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. Hydroxyl radical is the principal contributor for tissue injury of liver and any tissue. The (LRM, LRA & LRUSM) extracts of the arial part of *Leptadenia reticulata* showed promising free radical scavenging effect of DPPH in a concentration dependant manner. The results of scavenging effect of different extracts of the arial part of *Leptadenia reticulata* on DPPH radical are given in Fig 2. It indicated that

The LRA extract showed more reduction with the stable free radical DPPH to the yellow-colored Diphenylpicryl-hydrazyl than LRM and LRUSM extracts. The reference standard ascorbic acid (Vitamin-C) and BHA also demonstrated a significant radical scavenging potential in the concentration of 20-100 µg / ml. The extracts of *Leptadenia reticulata* plants were considered to play important roles in the prevention of number of degenerative diseases like Hepatic disorders, immune dysfunction, cataracts and macular degeneration by inhibiting the ROS production which may lower the risk and allows for proper functioning of the organs.

**Conclusion**

Thus from the present study it can be concluded that the different extracts of the *L. Reticulata* stem exhibited a significant phenolic content with promising free radical scavenging effect of DPPH in a concentration dependant manner. The LRA showed more total phenolic content, hence more scavenging activity than the LRM and LRUSM extracts which contain Antioxidant compounds like phenolic acids, polyphenols and

flavonoids scavenge free radicals such as peroxide; hydroperoxide or lipid peroxyl which are thereby involved in reducing the risk of diseases associated with oxidative stress help to show a synergetic effect on hepatoprotective activity. Identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine. Antioxidant compounds in food play an important role as a health-protecting factor and it neutralizes the free radicals, which are unstable molecules and are linked with the development of a number of degenerative diseases and conditions including hepatic disease, immune dysfunction, cataracts and macular degeneration. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases and conditions.

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**Fig. 1:** Standard Plot of Total Phenolic Content.

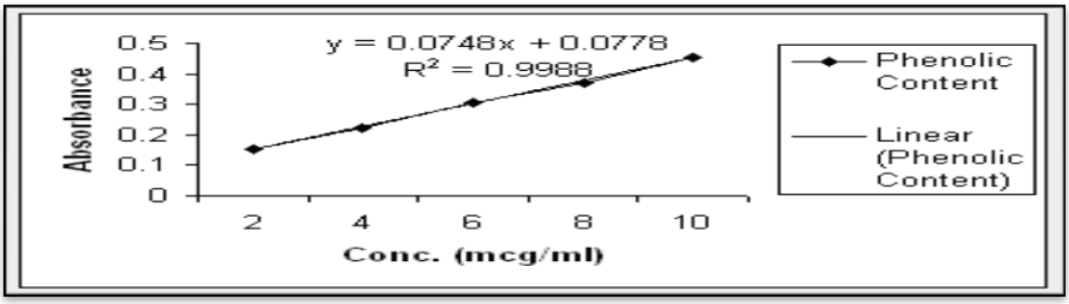


**Fig. 2:** Free radical scavenging activity by DPPH.

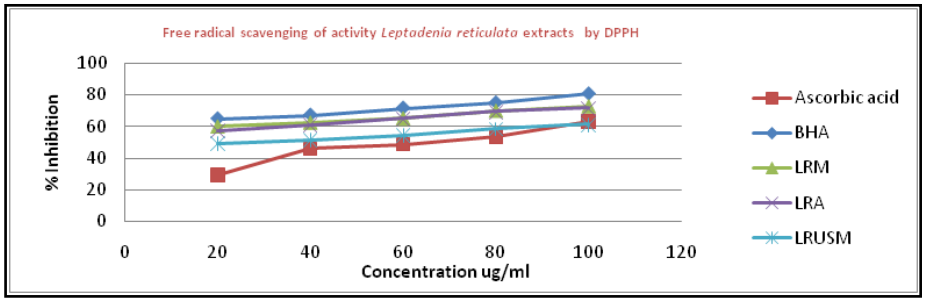

**Table 1:** TPC and TFC & % Inhibition of DPPH free radical by different extracts of *Leptadenia reticulata.*

| Plant name | Extracts OL | Yield of extracts | TPC value (Eq to GAA) | IC50 (µg/ml) |
|---|---|---|---|---|
| ***Leptadenia reticulata*** | Acetone Extract(LRA) | 8.01% w/w | 2.77 % | 55.55 |
| | Methanol Extract(LRM) | 12.11% w/w | 1.33 % | 56.66 |

**Table 2:** Total phenolic content of *L. Reticulata.*

| Conc.(µg/ml) | Absorbance | | | Mean |
|---|---|---|---|---|
| Gallic acid | I | II | III | |
| 2 | 0.154 | 0.156 | 0.152 | 0.154 |
| 4 | 0.224 | 0.228 | 0.221 | 0.224 |
| 6 | 0.302 | 0.308 | 0.31 | 0.307 |
| 8 | 0.374 | 0.371 | 0.372 | 0.372 |
| 10 | 0.452 | 0.456 | 0.454 | 0.454 |
| *LRM* | 0.283 | 0.285 | 0.288 | 0.285 |
| *LRA* | 0.185 | 0.184 | 0.165 | 0.178 |

**Table 3:** Free radical scavenging activity by DPPH.

| Sr. No. | Conc. (µg/ml) | Absorbance | | | Mean ± SEM | % Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | | | |
| Control | | 0.478 | 0.455 | 0.465 | 0.466 ± 0.002 | -- | -- |
| **Standard (Ascorbic acid)** | | | | | | | |
| 1 | 20 | 0.332 | 0.327 | 0.335 | 0.331 ± 0.002 | 28.88 ±0.829 | **40.70 (µg/ml)** |
| 2 | 40 | 0.249 | 0.257 | 0.252 | 0.253 ± 0.002 | 45.73 ± 1.268 | |
| 3 | 60 | 0.237 | 0.243 | 0.239 | 0.240 ± 0.002 | 48.53 ± 1.103 | |
| 4 | 80 | 0.211 | 0.225 | 0.217 | 0.218 ± 0.004 | 52.87 ± 1.861 | |
| 5 | 100 | 0.155 | 0.175 | 0.185 | 0.172 ± 0.009 | 63.10 ± 2.266 | |
| **Standard (BHA)** | | | | | | | |
| 1 | 20 | 0.159 | 0.164 | 0.168 | 0.164 ± 0.003 | 64.64 ± 0.730 | **61.74 (µg/ml)** |
| 2 | 40 | 0.152 | 0.157 | 0.149 | 0.153 ± 0.002 | 67.21 ± 0.865 | |
| 3 | 60 | 0.132 | 0.130 | 0.134 | 0.132 ± 0.001 | 71.80 ± 0.294 | |
| 4 | 80 | 0.110 | 0.119 | 0.120 | 0.116 ± 0.003 | 75.00 ± 0.993 | |
| 5 | 100 | 0.094 | 0.089 | 0.086 | 0.090 ± 0.002 | 80.75 ± 0.374 | |
| **Methanolic extract of *Leptadenia reticulata (LRM)*** | | | | | | | |
| 1 | 20 | 0.191 | 0.187 | 0.183 | 0.187 ± 0.002 | 59.86 ± 0.510 | **56.66 (µg/ml)** |
| 2 | 40 | 0.181 | 0.176 | 0.170 | 0.176 ± 0.003 | 62.30 ± 0.620 | |
| 3 | 60 | 0.168 | 0.164 | 0.157 | 0.163 ± 0.003 | 65.02 ± 0.663 | |
| 4 | 80 | 0.148 | 0.143 | 0.130 | 0.140 ± 0.005 | 69.88 ± 1.088 | |
| 5 | 100 | 0.132 | 0.127 | 0.122 | 0.127 ± 0.003 | 72.74 ± 0.517 | |
| **Acetone extract of *Leptadenia reticulata (LRA)*** | | | | | | | |
| 1 | 20 | 0.210 | 0.197 | 0.191 | 0.199 ± 0.006 | 57.22 ± 0.867 | **55.55 (µg/ml)** |
| 2 | 40 | 0.185 | 0.189 | 0.173 | 0.182 ± 0.005 | 60.84 ± 1.269 | |
| 3 | 60 | 0.161 | 0.168 | 0.163 | 0.164 ± 0.002 | 64.77 ± 0.939 | |
| 4 | 8 | 0.154 | 0.148 | 0.143 | 0.144 ± 0.002 | 69.16 ± 0.546 | |
| 5 | 100 | 0.132 | 0.139 | 0.127 | 0.133 ± 0.003 | 71.50 ± 1.030 | |
| **Unsaponifiable matter of *Leptadenia reticulata (LRUSM)*** | | | | | | | |
| 1 | 20 | 0.243 | 0.237 | 0.230 | 0.237 ± 0.004 | 49.20 ± 0.757 | **47.20 (µg/ml)** |
| 2 | 40 | 0.231 | 0.226 | 0.221 | 0.226 ± 0.003 | 51.48 ± 0.627 | |
| 3 | 60 | 0.219 | 0.211 | 0.207 | 0.212 ± 0.004 | 54.17 ± 0.506 | |
| 4 | 80 | 0.197 | 0.194 | 0.181 | 0.193 ± 0.002 | 60.02 ± 0.668 | |
| 5 | 100 | 0.187 | 0.179 | 0.175 | 0.180 ± 0.004 | 61.58 ± 0.431 | |

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