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QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES AND ANTIOXIDANT POTENTIAL OF *LEPTADENIA RETICULATA* (RETZ.) WIGHT & ARN LEAF EXTRACTS

Roli Shukla*

Department of Chemistry, Govt. MLB, Bhopal (M.P.)

***Correspondence Info:**

Dr. Roli Shukla
Professor, Depatment of Chemistry,
Govt. MLB College, Bhopal (M. P.)
Email:
k2pavni@gmail.com

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ABSTRACT

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various diseases. Jivanti (*Leptadenia reticulata* (Retz.) is a well known climber used for its innumerable therapeutic properties like antioxidant, antibacterial, vasodilator, galactogogue, Jivaniya etc., Its use in veterinary practice is tremendous due to its lactogenic effect. The aim of the present study was to determine qualitative and quantitative phytochemical and in vitro antioxidant activities of leaf of *L. reticulata* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of flavonoids was carried out by aluminium chloride method. The *in vitro* antioxidant activity of hydroalcoholic extract of the leaf was assessed against DPPH assay method using standard protocols. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids. The total flavonoids content of leaves hydroalcoholic extract was 1.943mg/100mg. The activities of hydroalcoholic leaves extract against DPPH assay method were concentration dependent with IC 50 values of ascorbic acid and extracts 14.11and 68.88µg/ml respectively. These studies provided information for standardization and correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Key words: *Leptadenia reticulata,* Qualitative, Quantitative phytochemical, Antioxidant

INTRODUCTION:

India is a rich source of medicinal plants and a number of plant derived oils and extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes and alkaloids (Osawa et al., 1990; Keith et al., 1990) have received considerable attention in recent years due to their diverse pharmacological properties. The qualitative

analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques (Sneader, 2000). Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available (Aguoru et al., 2014). Reactive Oxygen Species (ROS), such as hydrogen peroxide, super oxide anion and hydroxyl radical, capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age (Beris, 1991). Antioxidant that scavenges these free radicals proves to be beneficial for these disorders as they prevent damage against cell proteins, lipids and carbohydrates (Marnett, 2000). Erythrocytes, which are the most abundant cells in the human body, possessing desirable physiological and morphological characteristics are exploited extensively in drug delivery (Hamidi, 2003). Oxidative damage to the erythrocyte membrane (lipid/ protein) may be implicated in haemolysis associated with some haemoglobinopathies, oxidative drugs, transition metal excess, radiation, and deficiencies in some erythrocyte antioxidant systems (Hsiao et al., 1997).This assay is useful either for screening studies on various molecules and their metabolites, especially on one hand, molecule having an oxidizing or antioxidating activity or on the other hand, molecule having a long term action (Djeridane et al., 2007). Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage (Kumar et al., 2013). These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation (Yu, 2001; Ebrahimzadeh et al., 2010). *L. reticulata* belongs to family Asclepiadaceae is an important medicinal plant. This plant is commonly known as Jivanti or Dori or Swarn, and is distributed in tropical and sub-tropical parts of Asia and Africa. In India, it is found in Gujarat, sub - Himalayan tracts from Punjab to Sikkim and Khasi hills and throughout peninsular India (Rajeswari, 2014). Jivanti is jeevana tonic that boosts energy level of the body, according to ayurveda. It is beneficial for the patient who suffers from weak debility or a lack of energy. It also increases longevity, memory enhancement, immunomodulation and adoption. Lactogenic, anabolic and galactogogue effect was also observed in it. Plant is also of great value in tridoshas (Vatta, Pitta and Kapha) (Kaushik and Joshi, 2013). Important constituents present in it like carbohydrates, steroids, coumarins, flavonoids, tannins, phenolics and alkaloids (Chetankumar et al., 2012). the major phytochemical compound is stigmasterol. It also contains ß–sitosterol α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, stigmasterol, hentriacontanol, a triterpene alcoholsimiarenol and apigenin (Kaushik and Joshi, 2013). The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and in vitro antioxidant activity of leaf of *L. reticulata*.

MATERIAL AND METHOD

Plant material

The leaf of *L. reticulata* was collected from local area of Bhopal (M.P.) in the month of December, 2018. Plant material (leaves)

selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Extraction

Dried powdered leaves of *L. reticulata* has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening of the extract

The extract of *L. reticulata* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids (Khandelwal; 2005; Kokate; 1994).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso *et al* .2011. one ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method [18]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of

control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

RESULTS AND DISCUSSIONS

The percentage yields of hydroalcoholic extract obtained from *L. reticulata* are depicted in the table 1. Preliminary phytochemical studies of the extract were done according to the published standard methods. These tests were broad in scope and used to determine the presence of flavonoids and saponins but all constitute was absents in the extract Table 2. The total flavonoid content of the extracts of was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extract of *L. reticulata* showed the content values of 1.943. Results are provided in Table 3 and Fig. 1.

Table 1 % Yield of hydroalcoholic extract

S. No.	Plant	% Yield (w/w)
1	*Leptadenia reticulata*	5.75

Table 2 Result of Phytochemical screening of hydroalcoholic extracts

S. No.	Constituents	*Piper betle*
1.	Alkaloids	-ve
2.	Phenol	-ve
3.	Flavonoids	+ve
4.	Carbohydrate	-ve
5.	Proteins	-ve
6.	Saponins	+ve

Table 3 Total flavonoid content of *Piper betle*

S. No.	Extracts	Total flavonoid (QE) (mg/100mg)
1.	**Hydroalcoholic**	1.943

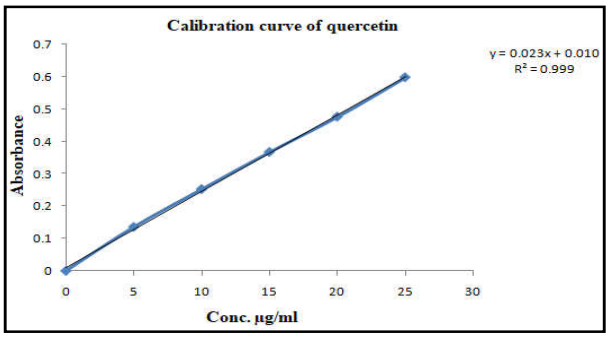

Fig. 1 Graph of Estimation of Total flavonoid content

Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 20 µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 4 and Fig.2

Table 4 % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

Concentration (µg/ml)	Ascorbic acid % Inhibition	Extract % Inhibition
10	40.92	20.06
20	48.70	27.93
40	73.48	30.70
60	89.04	46.75
80	90.20	51.85
100	91.35	69.44
IC 50	14.11	68.88

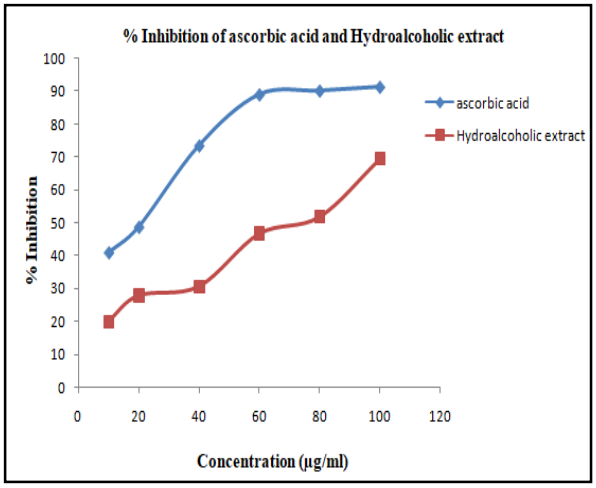

Fig.2 Graph of % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

Conclusion

It can be concluded that from present investigation the phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The total flavonoid content in hydroalcoholic leaves extract is further proved by in vitro antioxidant studies. Potential antioxidant activity has good correlations with the therapeutic use in the treatment of cardiovascular disorders. Further research to isolate individual compounds, their *in-vivo* antioxidant activities with different mechanism is needed.

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