ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 8, Issue, 11, pp.41565-41570, November, 2016*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

## RESEARCH ARTICLE

# STUDY ON DISTRIBUTION, PROPAGATION, PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF AN ENDEMIC LIANA *BEAUMONTIA JERDONIANA* WIGHT

**[1]Joseph John, *[,2]Sujana, K. A. and [1]Anil Kumar, N.**

[1]M. S. Swaminathan Research Foundation, Puthoorvayal, Meppadi P. O., Kalpetta, Wayanad, Kerala, India- 673577

[2]Central Botanical Laboratory, Botanical Survey of India, AJCB Indian Botanic Garden, Botanical Garden P. O., Howrah, West Bengal, India – 711103

**ARTICLE INFO**

*Article History:*
Received 20th August, 2016
Received in revised form
22nd September, 2016
Accepted 15th October, 2016
Published online 30th November, 2016

*Key words:*

Antimicrobial activity,
*Beaumontia jerdoniana*,
Distribution,
Endemism,
Liana,
Phytochemicals.

**ABSTRACT**

This study investigated in levels both the field and laboratory during the period of 2006 to 2014. Detailed distribution, phenology, taxonomy and propagules of the plant collected from natural population and further research carried out from the plants growing in RET plant conservatory of M. S. Swaminathan Botanic Garden. The screening of phytochemical constituents, proximate analysis, antioxidant, antimicrobial properties of methanolic leaf extracts of *Beaumontia jerdoniana* Wight were done and results tabulated here. The results of this study indicate that leaf extracts of *B. jerdoniana* exhibits antioxidant, anti-microbial activities which explain its use in animal nutrition and human medicine. Among the thirteen qualitative phytochemical tests conducted nine showed presence and four showed absence. Quantitative determination of ten phytochemicals were conducted and found that the plant contain good amount of tested phytochemicals in which Flavonoids ranked highest with 0.105±0.004mg/ml followed by Alkaloids 0.94±0.03mg/ml. Proximate nutritional composition also was tested and found that quantity of Carbohydrates were highest with 0.133±0.004mg/ml while other constituents like protein and ash composition also present in various quantities. The antimicrobial activity against selected five microorganisms were tested and found that the extract showed highest inhibition against *Staphylococcus aureus* followed by *Escherchiacoli* and *Kebsiella pneumonia*. The study showed the importance of man assisted conservation for this important plant where in situ and ex-situ conservation strategies have to be adopted.



**Citation: Joseph John, Sujana, K. A. and Anil Kumar, N. 2016.** "Study on distribution, propagation, Phytochemical and antimicrobial screening of an endemic liana *Beaumontia jerdoniana* Wight", *International Journal of Current Research*, 8, (11), 41565-41570.

## INTRODUCTION

Endemism is an important concept in conservation biology. Plants can be endemic to all kinds of features and geographic areas, ranging from mountain peaks, mountain ranges, river basins, and watersheds to political boundaries such as parks, preserves, counties, and states and physical attributes like soils and rock types. Endemic species must rely exclusively for their long-term viability and continued existence on the management of the geographical area to which they are restricted. Endemism is one of the criteria used to setpriorities for species conservation efforts.*Beaumontia jerdoniana* Wight (Apocynaceae) is an endemic liana narrowly distributed to several pockets of Western Ghats. Very limited information on the distribution, propagation and the properties like antimicrobial activity, phytochemical constituents was available on this species. Knowledge on distribution, germination and establishment, rarity, and usefulness of the plants will pave the way for formulating plan for people participatory conservation efforts. On this ground this work intend to document distribution, propagation and screening of phytochemical and antimicrobial properties.

***Corresponding author: Sujana, K. A.***
Central Botanical Laboratory, Botanical Survey of India, AJCB Indian Botanic Garden, Botanical Garden P. O., Howrah, West Bengal, India – 711103.

## MATERIALS AND METHODS

### Field studies

The study was conducted during the period of 2006-2014. As an initial step of research, the regional Floras were studied to make correct taxonomic identification of the target species and to locate the extension of distribution of plant species. Further the species were familiarized by examining specimens at MH, CALI, CAL, WRC, KFRI and HIFP. With the help of forest officials and local inhabitants surveyed the interior forest areas to locate the natural populations in already reported localities

as well as other similar habitats and phenology was documented after repeated visits. Further, the healthy mother populations were identified for conducting propagation trials. The extent of natural regeneration, the seed characteristics, and mode of germination were studied using standard techniques and suitable propagation measures were formulated to multiply the species. The plants propagated in nursery planted in RET plant conservatory of M. S. Swaminthan Botanic Garden, MSSRF, Wayanad, Kerala for further research and conservation.

**Collection and preparation of sample**

The fresh leaves of *B. jerdoniana* collected from M. S. Swaminathan Botanic Garden of M. S. Swaminathan Research Foundation, Kalpetta, Kerala, India. The fresh sample of about 1 kg were collected in polythene bags and taken to the laboratory. The leaves were surface sterilized and washed with clean sterile water. Then the leaves were shade dried until all the water molecules evaporated and dried for one hour at 160°C. After drying the leaves were ground well using mechanical blender into fine powder and then transferred into air tight container.

**Preparation of Extract**

Plant materials were washed with clean sterile water and shade dried and powdered using mortar and pestle. 10g of dried sample was taken in 100ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker for 24 hours. After 24 hours, the supernatant was collected and the solvent was evaporated to make the final volume (one fourth of original volume) and stored at 4°C in air tight bottles.

**Phytochemical screening**

Different qualitative and quantitative tests were performed for establishing the profile of phytochemicals of the leaves. The extracts were subjected to experiments such as alkaloids, flavonoids, tannins, phenolic compounds, cardiac glycosides, terpenoids, anthraquinones, saponins, and steroids in accordance with the procedures mentioned by Benavente-Garcia *et al*., 1997; Marjorie 1999; Ali *et al*., 2008.

**Proximate Nutritional analysis**

Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemistand Harborne (1980) and Kessler *et al*. (2003). This constitutes the class of food present in samples such as carbohydrate, protein, fat, crude fiber, ash content and moisture content.

**Antibacterial activity**

Paper discs impregnated with specific antibiotics for the test substances are placed on the surface of the Nutrient agar medium inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard. The plates are incubated and the zones of inhibition around each disc were measured. Cultures of microorganism were collected from the laboratory of CAbC MSSRF. The overnight culture of pathogenic test organisms swabbed on to Nutrient Agar medium. Different dilutions of five plant extracts were prepared in the order of 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml respectively. 10µl of each concentration was introduced into each filter disc (5mm in diameter) and discs were placed in one plate. The plate was incubated at 37°C for 24 hours and zone of inhibition was measured. The inhibition zone of plant extracts were compared with antibiotic ampicillin. The activity index of each plant extract was calculated by using the formula.

$$\text{Activity index} = \frac{\text{Inhibition area of sample}}{\text{Inhibition area of standard}}$$

**Antifungal assay**

Potato Dextrose Agar medium with different concentrations of 20%, 40% and 60% of the methanolic extracts of the test plants was prepared. About 15 ml of the medium was poured into each petriplate and allowed to solidify. Cultures of selected fungi were taken from the laboratory of CAbC MSSRF. Disc with size of 5mm 7-day-old culture of the test fungi were placed at the center of the petriplates and incubated at 25±2 ° C for seven days. After incubation the colony diameter was measured in millimeter. For each treatment four replicates were maintained. PDA medium without the methanolic extract served as control. The fungitoxicity of the extracts in terms of percentage inhibition of mycelial growth was calculated by using the formula.

$$\text{Percentage of inhibition} = \frac{\text{dc} - \text{dt}}{\text{dcx100}}$$

where dc = Average increase in mycelial growth in control
dt = Average increase in mycelial growth in treatment.

**Statistical Analysis**

Each assay in this experiment was repeated thrice and the values were expressed as mean of triplicate analysis of the samples (n=3) ±standard deviation (SD).

## RESULTS AND DISCUSSION

**Taxonomic Description of *Beaumontia jerdoniana* Wight**

Woody climbers; branchlets pale brownish, terete, 3-10 mm thick, glabrous. Leaves opposite, 12-25 × 3.5-12 cm, narrowly oblong, oblong-elliptic to obovate, obtuse to rounded at base, entire, abruptly caudate at apex (cauda 5-10 mm long), thinly coriaceous, glabrous, dark brown above when dry, paler beneath; lateral nerves 8-14 pairs, faint above, prominent beneath, lax, arcuate; nervules faint above, prominent beneath, scalariform, mostly branched; petioles 1-4 cm long. Inflorescence terminal, few- flowered subumbellate cymes, often once branched towards apex, rusty-pubescent, up to 15 ×15 cm; peduncles 1-4 cm long; bracts foliaceous, up to 15 mm long. Flowers white; pedicels 2.5-3.5 cm long, densely adpressed tawny- pubescent. Calyx lobes 5, foliaceous, almost free, elliptic-lanceolate to oblong-lanceolate, 10-15 × 4-5 mm, acute, densely tawny pubescent. Corolla campanulate; tube ca 15 mm long, hairy at throat inside, lobes 5, spathulate-obovate, 6-7 × 3-3.5 cm, evanescently puberulous outside, glabrous inside. Stamens 5, inserted on the corolla tube; filaments intertwisted; anthers sagittate, adhering with the stigma. Ovary 1, 2-locular; style filiform, puberulous; stigma oblong-fusiform.Fruits splitting into 2 follicles at full maturity, ca 25 cm long, thick and woody.

**Phenology:** December – May

**Distribution:** Endemic to Western Ghats

***Maharashtra*:** Pune, Ratnagiri
***Karnataka*:** Coorg, Hassan, Mysore, North Kanara
***Kerala*:** Wayanad, Kasaragod, Kannur, Kozhikode
***Tamilnadu*:** Nilgiri

**Propagation measures and Ornamental potential**

*Beaumontia jerdoniana* Wight, a narrow endemic species of Western Ghats attracts its special attention because of its fragrant beautiful flowers. It is a gorgeous liana covered with green leathery leaves. This fast growing plant produces large, white, aromatic trumpet-shaped flowers during December – January. Its fruits are long thick and woody, at length separating into spreading follicular mericarps. Since the plant shows unrestrained growth, a large conservatory or shade is necessary for its cultivation (Fig.1). It will require a rich soil, well-drained soil and plenty of water over the growing season. Be aware though that it dislikes heavy, wet soil in monsoon and can suffer severe root damage if kept water-logged over this period. If heavy soil is difficult to avoid then create a raised bed and add plenty of grit and organic matter to improve the drainage. Planting should be done just before rainy season. While planting, wires or strings should be attached as support so that the shoots can hold up and climb. The side shoots need to be shortened by two thirds after the flower wither off, which results in the formation of new shoots. Cuttings of new young shoots can be inserted in sand or sandy soil bed or tray and place them in a propagating chamber until they get rooted.

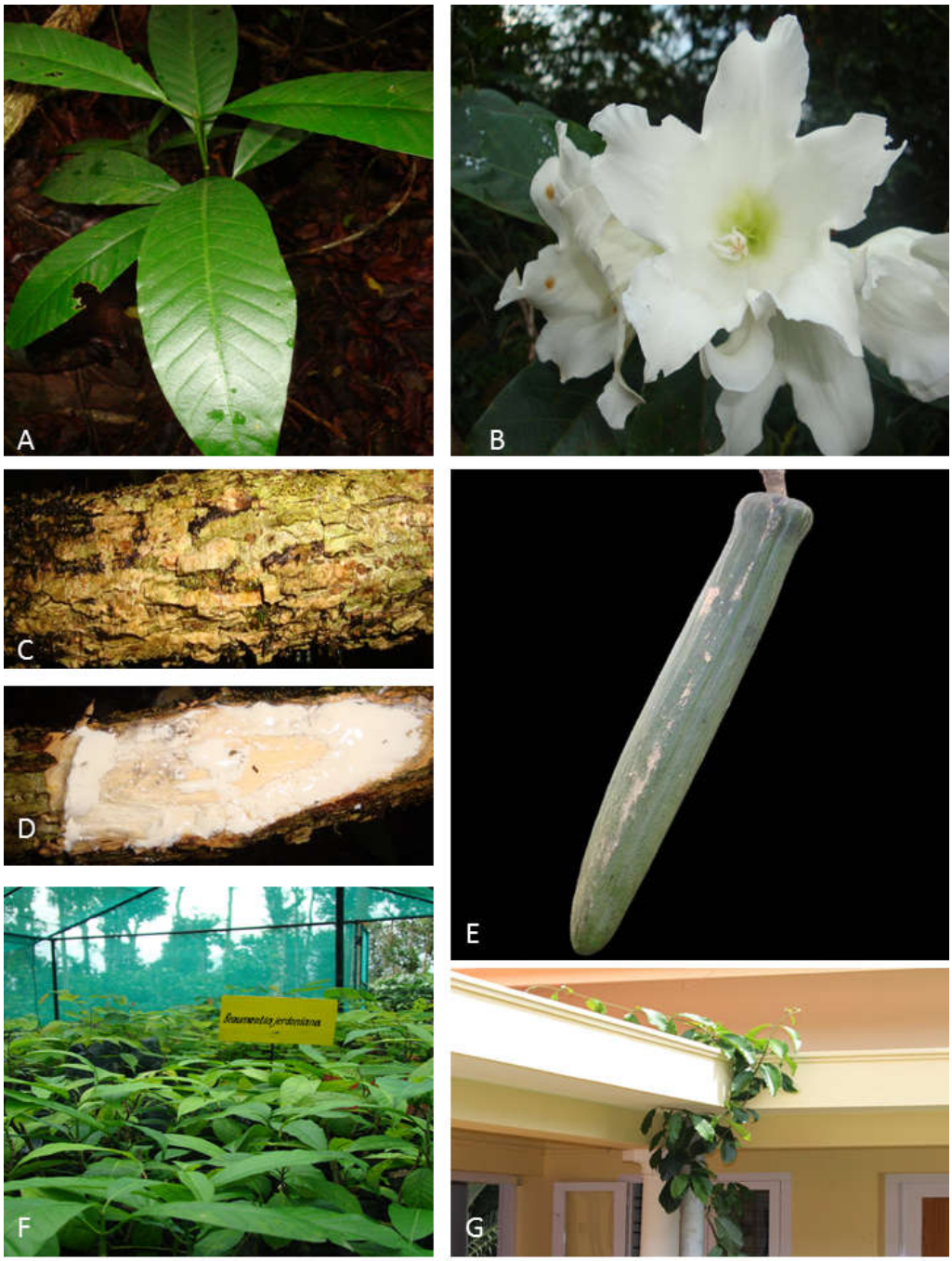


**Fig. 1. *Beaumontia jerdoniana* Wight – A. Habit, B. Flowers, C. Stem, D. Milky latex exudates from stem slash, E. Follicle, F. Seedlings growing in Propagation chamber, G. Plant growing in a patio**

They can then be potted in individually, in 5 ×6 inch pots containing equal parts of sand, farm soil and compost and subsequently in their permanent position. Seed germination is also possible to raise seedlings; seed planted in sand will give rise to new healthy seedlings within 3 weeks. Its white flowers in terminal cymes and evergreen nature makes it quite show and it too has the potentiality to be introduced as an ornamental plant as *Beaumontia grandiflora* which is a close allied species of the same plant. Through this conservation effort, M. S. Swaminathan Research Foundation distributed 1200 seedlings to farmers, planters, environmentalists, schools and colleges through vine hut program for ex-situ conservation of RET woody climbers. Efforts were taken to introduce this species to landscape designers.

## Phytochemical analysis

Screening of phytochemicals of the leaf extracts of *B. jerdoniana* revealed the presence of acidic compounds, phenols, flavonoids, alkaloids, tannins, glycosides, steroids, carbohydrates and reducing sugars (Table 1).

**Table 1. Qualitative determination of Phytochemicalsin themethanolic extracts of leaves of *Beaumontia jerdoniana***

| Phytochemical components | Status |
|---|---|
| Acidic compounds | + |
| Phenols | + |
| Flavonoids | + |
| Alkaloid | + |
| Tannins | + |
| Glycosides | + |
| Saponins | – |
| Terpenoids | – |
| Steroids | + |
| Antraquinones | – |
| Carbohydrates | + |
| Reducing sugar | + |
| Resins | – |

+ = present; - = absent

**Table 2. Quantitative determination of Phytochemicals in *Beaumontia jerdoniana***

| Components | Quantity (mg/ml) |
|---|---|
| Total phenolic content | 0.018+0.002 |
| Flavonoids | 0.105±0.004 |
| Tannic acid | 0.028±0.002 |
| Alkaloids | 0.94±0.03 |
| Total carbohydrates | 0.13±0.003 |
| Reducing sugar | 0.044±0.005 |
| Inulin | 0.32±0.003 |
| Sucrose | 0.122±0.003 |
| Cellulose | 0.05±0.003 |
| Ascorbic Acid | 0.13±0.004 |

**Table 3. Proximate nutritional composition of leaves of *Beaumontia jerdoniana***

| Plant materials | Moisture (%) | Total solids (%) | Crude Ash (%) | Crude Fiber (%) | Protein (mg/ml) | Amino acid (mg/ml) | Carbohydrate (mg/ml) |
|---|---|---|---|---|---|---|---|
| *Beaumontia jerdoniana* | 70±1 | 30.33±1.52 | 2.46±0.35 | 36.23±0.37 | 0.045±0.004 | 0.046±0.00 | 0.133±0.004 |

**Table 4. Antioxidant activity of leaves of *Beaumontia jerdoniana***

| Total antioxidant capacity (%) | Enzymatic antioxidants | | Non enzymatic antioxidants | | |
|---|---|---|---|---|---|
| | Catalase (%) | Peroxidase (%) | NO radical scavenging activity (%) | $H_2O_2$ Scavenging activity (%) | Reducing power (%) |
| 52.2±0.26 | 59.33±0.04 | 39.23±0.03 | 7.64±0.04 | 45.17±0.03 | 68.03±0.35 |

**Table 5. Antibacterial activity of methanolic extract of leaves of *Beaumontia jerdoniana***

| Test microorganism | Inhibition zone of standard Ampicillin in mm | Inhibition zone (IZ) in mm | Activity index |
|---|---|---|---|
| *Escherichia coli* | 16 | 6 | 0.375 |
| *Klebsiellapneumoniae* | 16 | 6 | 0.375 |
| *Staphylococcus aureus* | 15 | 10.5 | 0.700 |
| *Enterobacteraerogens* | 16 | 6 | 0.375 |
| *Salmonella typhi* | 18 | 6 | 0.333 |

**Table 6. Antifungal activity of methanolic extract of leaves of *Beaumontia jerdoniana***

| Pathogen | Colony diameter in mm | | | | Mean diameter in test | Percentage of inhibition |
|---|---|---|---|---|---|---|
| | Control | Methanolic extract | | | | |
| | | 20% | 40% | 60% | | |
| *Aspergillusniger* | 15 | 7 | 6 | 6 | 6.33 | 57.8 |
| *Phytophthoracapsici* | 57 | 5 | 5 | 5 | 5 | 91.22 |
| *Fusariumoxysporum* | 12 | 5 | 5 | 5 | 5 | 58.33 |
| *Curvulariageniculata* | 31 | 6 | 5 | 5 | 5.33 | 82.80 |
| *Pythiumaphanidermatum* | 13 | 5 | 5 | 5 | 5 | 61.53 |

Quantitative determination of the phytochemicals present in this plant was also performed (Table 2). The proximate nutritional composition showed moisture was the highest (70±1%) while crude ash (2.46±0.35%) was the least and also analyzed protein (0.045±0.004mg/ml), amino acids (0.046±0.00mg/ml) and carbohydrate (0.133±0.004 mg/ml) (Table 3). The leaf of *B. jerdoniana* was tested for their antioxidant content, their ability to scavenge free radicals and their biomolecular protective effects. The results obtained are presented below in Table 4. The enzymatic antioxidants analyzed were catalase and peroxidase. Non-enzymatic antioxidants like NO radical scavenging activity, $H_2O_2$ scavenging activity and reducing power determination assessed as well.

**Antimicrobial activity**

The antimicrobial activity of the extracts of *B. jerdoniana*were studied against five pathogenic bacterial strains, namely *Escherichia coli, Klebsiellapneumoniae, Staphylococcus aureus, Enterobacteraerogenes* and Salmonella typhiand five fungal strains (*Aspergillusniger, Phytophthoracapsici, Fusariumoxysporum, Curvulariageniculata, Pythium aphanidermatum*). Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of growth. The antibacterial activity index of *B. jerdoniana* was found to be highest to against *Staphylococcus aureus* and least to *Salmonella typi* (Table 5). The fungicidal activities of the leaves of *B. jerdoniana* are provided in Table 6. The methanolic extract of the leaves were found to cause 91.22% inhibition of the growth of *Phytophthoracapsici* followed by 83.87% of inhibition of growth of *Curvulariageniculata* and more than 45% inhibition showed the remaining pathogens.

## DISCUSSION AND CONCLUSION

It is well known that anthropogenic activities during this century have clearly impacted many rare and endangered plants. Conservation guidelines, strategies, or plans for endemic and rare taxa in India should take into account. The land-use changes that are occurring for the near future also need to take considers. Knowledge of distribution patterns and usefulness is essential in determining the kinds of conservation activities necessary to prevent species extinction. Phytochemicals analyzed with the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007). Phenolics content are very important plant constituents because they can act as reducing agents, hydrogen donors and metal chelator (Parr and Bolwell, 2000). They also act as radical scavenger due to their hydroxyl groups. The total phenolic content of *B. jerdoniana* was 0.019mg/ml. Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, to copherolsetc (Ali *et al*., 2008). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation (Benavente-Garcia, 1997). Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie 1999). Flavonoids show their antioxidant action through scavenging or chelating process (Li and Zhou, 2007). More than 2000 flavonoids have been reported among woody and non-woody plants (Kessler *et al*., 2003). In this study, the flavanoid content of *B. jerdoniana* was 0.105mg/ml. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Harborne, 1980) and activity in the nervous system especially in the action towards neurotransmitters such as acetylcoline, epinephrine, norepinephrine, dopamine and serotonin(Nobori 1994). In the present study *B. jerdoniana* shows alkaloid content of 0.95 mg/ml. It is obvious that this plant can be considered as a resource for the mass extraction of alkaloids for industrial applications. The carbohydrates in food are of major interest in relation to chronic diseases. Different types of carbohydrates give rise to different glycaemic responses, and also able to stimulate lipogenesis Margaret and Michael (1993). Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid) have been incinerated at a temperature of about 500$^{o}$C. Ash content is generally taken to be a measure of the mineral content of the original food (Harborne, 1980). Least amount of Ash content was obtained for *B. jerdoniana* in this study. High percentage of moisture content, this is an indication that they possess large number of cell saps. Water is clearly the most important nutrient and the most abundant substance in the most of the living organisms. The proximate analysis showed the moisture content of *B. jerdoniana* was 70%. In this work, the selected liana species shows highest amount of crude fiber. Crude fibre in food is an indication of the level of non-digestible carbohydrate and lignin. Crude fibre is made up largely of cellulose together with a little lignin which is indigestible in human (Weisburger, 2001). Thus the presence of fibre may have role in providing shape to leaves and imparting health to the plant.

Antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is an increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants. The reducing power of bio active compound is generally associated with the presence of reductions which have been shown to exert antioxidant action by breaking the free radical chains by donating a hydrogen atom (Pin-Der Duh, 1998). The total antioxidant capacity of *B. jerdoniana* was 52%. Hydrogen peroxide is an important biological reactant because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell (Gulcin *et al*., 2003). In the present study *B. jerdoniana* have a moderate source of hydrogen peroxide scavenging activity. Scavenging of $H_2O_2$ by the plant extracts may be attributed to their phenolics, which donate electron to $H_2O_2$, thus reducing it to water. The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner.

Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxy nitrite which can be decomposed to form OH radical. The level of nitric oxide in *B. jerdoniana*was7.69%. Since NO plays a crucial role in the pathogenesis of inflammation (Moncada *et al*., 1991) this may explain the use of chosen liana species for the treatment of inflammation and for wound healing. Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal

failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism. Detailed investigations have to be made on the role of nitric oxide from this plant in the healing of wounds and against more clinical pathogens. The most common ROs molecule is $H_2O_2$, it is mainly produced by mitochondria as a byproduct of oxidative metabolism. Because a high level of $H_2O_2$ is cytotoxic and can modify the protein confirmation of alter protein function, the tissue of $H_2O_2$ scavenging is also important. In addition to ROs, NO is also implicated in several pathological conditions (Li and Zhou, 2007). Plant extracts have been reported to possess components acting as electron donors. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth. Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death (Kessler *et al*., 2003). As methanol extract of these plants showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant agent might be developed from these plants for the treatment of disorders associated with free radicals. Phenolic compounds containing free hydrogen are largely responsible for antioxidant activity (Evans *et al*., 1996), thus the phenol compounds of *B. jerdoniana*can be referred to be responsible for the antioxidant activity.

The presences of alkaloids and phenolic compounds are thought to be toxic to microorganisms, inhibiting the enzymes which are essential for the growth of microorganisms. Thus there has been a continuing search for new and more potent antibiotics. In this work plant extracts found to be bactericidal and fungicidal properties. Therefore, this work can be an indication that its potential as a drug that can be used against these microorganisms. According to WHO on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence, the last decade has witnessed an increase in the investigations on plants as a source of human disease management. The results acquired in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be a valuable reservoir of bioactive compounds of substantial medicinal merit which we can be commercially exploited. Wayanad district of Western Ghats remains one of the botanical havens of India. New plant species are still being discovered in this range, and land users and managers across this magnificent landscape need to be aware of the unique biodiversity of Wayanad. Policy makers have to consider the evolutionary forces that have contributed to such a remarkable rare and endemic flora and provide appropriate levels of conservation to ensure that this resource has to be sustained.

## REFERENCES


Ali S.S., Kasoju N., Luthra A., Singh A., Sharanabasava H., Sahuand A., and Bora U. 2008. Indian medicinal herbs as source of antioxidants. *Food Res. Intl*., 41: 1-15.

Benavente-Garcia O., Castillo J., Marin F.R., Ortuno A. and Del-Rio J.A. 1997. Uses and properties of Citrus flavonoids. *J. Agri. Food Chem*., 45: 4505-4515.

Evans R.C.A, Miller N. J. and Paganga G. 1996.Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2: 152.

Gulcin I., Oktay M., Kirecci E. and Kufrevioglu O. I. 2003. Screening of antioxidant and Antimicrobial activities of anise (*Pimpinellaanisum* L.) seed extracts. *Food Chem*., 83: 371-382.

Harborne J. B. 1980. Plant phenolics, In: Bell E.A., Charlewood B.V. (Eds.), Secondary plant products, Berlin, Verlag, Springer. 320-322

Kessler M., Ubeaud G. and Jung L. 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol.,* 55: 131-142.

Li X.L. and Zhou A.G. 2007. Evaluation of antioxidant activity of the polysaccharides extracted from *Lyciumbarbarum* fruits in vitro. *European Polymer Journal*, 43: 488.

Margaret F. R and Michael W. 1993. Alkaloids Biochemistry, Ecology and Medicinal Applications, Plenum press, New York and London. 1-8.

Marjorie M.C. 1999. Plant, products as antimicrobial agents. *American Society for Microbiology*, 4: 564-582.

Moncada S., Palmer R.M.J. and Higgs E. A. 1991. Nitric oxide : Physiology, Pathophysiology and Pharmacology. *Pharmacol. Rev*., 43: 109-142.

Nobori T., Miurak K., Wu D.J., Takabayashik L.A. and Carson D.A 1994. Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*, 46: 753-756.

Parr A. and Bolwell G.P. 2000. Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agri.,* 80: 985-1012.

Pin-Der Duh X. 1998. Antioxidant activity of Budroock (*Arctiumlapp* Linn.): its scavenging effect on free radical and active oxygen. *J. Am. Oil Chem. Soc*., 75: 455-461.

Singh R., Singh S.K., and Arora S. 2007.Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food Chem. Toxicol*., 45: 1216-1223.

Sofowora A. 1993. Medicinal plants and traditional medicine in Africa, New York, John Wiley and Sons, 191-289.

Weisburger J. H. 2001. Eat to live, not live to eat, *Nutrition*. 16: 767-773.


*******