INTERNATIONAL RESEARCH JOURNAL OF PHARMACY ISSN 2230 – 8407
Available online www.irjponline.com Research Article

PHYTO-PHARMACOLOGICAL INVESTIGATIONS OF *CLERODENDRUM INFORTUNATUM* GARTN.

Rajurkar B. M.*
Assistant Professor in Botany, R. S. Bidkar College, Hinganghat Dist. Wardha, Maharashtra, India

Article Received on: 10/09/11 Revised on: 29/10/11 Approved for publication: 09/11/11

*Email: bmrajurkar@gmail.com

ABSTRACT
Clerodendrum infortunatum Gartn. is an important Indian medicinal plant and widely used in Ayurveda and siddha for management of various diseases. This aims a comprehensive of the chemical constituents, pharmacological and clinical uses. Different pharmacological experiments in both in vitro and in vivo have been carried out and also identified the medicinally important phyto-constitutents. A number of biological constituents in good yield and some have been shown to possess useful biological actions belonging mainly to Phenolics, Flavonoids, Steroids, Terpenoids, Fixed oil and Sugars. Extracts and chemical constituents of this plant possess useful pharmacological activities. The main pharmacological activity of *Clerodendrum infortunatum* Gartn. is wound healing effect, anti-venom property and anti-fertility activity. Many pharmacological studies of *Clerodendrum infortunatum* Gartn. have been demonstrated for their antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, wound healing effect, anti-venom and anti-fertility activities, supporting its traditional uses. Suggest a wide range of clinical applications for the treatment of anthelmintic, cathartic, diuretic, emetic, expectorant. Hence, this article contributes to the knowledge of *Clerodendrum infortunatum* Gartn. plant and their ethnopharmacological uses.
Keywords: *Clerodendrum infortunatum* Gartn., Biological constituents, Ayurveda, Therapeutic uses.

INTRODUCTION

The plant *Clerodendrum infortunatum* Gartn. is commonly known as Saraswaty's leaf and it belongs to the family Verbenaceae. The common names of *Clerodendrum infortunatum* Gartn. are Bhandika, bhantaka, bhargi (Sanskrit), Bhant (Hindi),Bhandira (Marathi). It is a common perennial shrub found mostly in forest areas of Tadoba National Park of Chandrapur District of Maharashtra State.

Synonyms

Sanskrit : Bhandika, bhantaka, bhargi
Hindi : Bhant
Marathi **:** Bhandira

Morphology

It is large shrubs or small trees about 4 m tall. Branchlets are quadrangular, fulvous and tomentose. The leaves simple, opposite, decussate; petiole 2.5-8 cm long , apex acuminate, base cordate, margin entire or dentate, subcoriaceous, fulvous tomentose; midrib raised with tomentose above; 5-7-nerved at base; secondary nerves about. 2-3 pairs; tertiary nerves distantly and horizontally percurrent. Inflorescence terminal panicle, flowers zygomorphic, white. Fruit is drupe black, nearly globose seated on enlarged pinkish accrescent calyx.

Chemical constituents

The major group of chemical constituents present in *Clerodendrum* genus are phenolics, flavonoids,terpenoids and steroids.

a. Phenolics: Acetoside, fumaric acid, methyl and ethyl esters of caffeic acid
b. Flavonoids: Apigenin, acacetin and methyl esters of acacetin-7-o-glucuronide, cabruvin, quercetin, scutellarein, scutellarein-7-o-β-D-glucuronide, hispidulin.
c. Steroids: Clerodolone, Clerodone, Clerodol and a sterol Clerosterol.
d. Terpenoids: Clerodin (saponin diterpenoid)
e. Fixed oil: Glycerides of Lenoleic, oleic, stearic and lignoceric acid.
f. Sugars**:** Raffinose, lactose, maltose, sucrose, glactose, glucose and fructose.

Parts Used

Leaves and root extract is useful for the treatment of fresh wound, piles, externally for tumours and certain skin diseases, and internally as tonics. Leaves are used as bitter tonic, vermifuge, laxative and cholagogue. Fresh leaf juice is introduced in the rectum for the removal of ascarids.

Uses

The leaves and root of the plant are employed externally for tumours and certain skin diseases, and internally as tonics. The root is useful in venereal and scrofulous complaints. In Indian homeopathy, it is used as a remedy for diarrhea, post natal care, and also to dress fresh wounds. Leaves are used as bitter tonic, vermifuge, laxative and cholagogue. Fresh leaf juice is introduced in the rectum for the removal of ascarids. Leaves and roots are used for external applications on tumours. The juice of the leaves is also believed to possess distinct anthelminitic properties.

Pharmacological Studies

The Sprague-Dawley rats (male) of weight 250-300 g were employed in this investigation. They were housed under the standard conditions of 22±3^{0}C, humidity 35% to 60% and artificially lighting was maintained for 12:12 h light:dark cycle. The animals were provided with regular rat chow and distilled water *ad libitum.*

Chemicals

Streptozotocin (Calbichem Darmstadt Germany) was used as standard diabetes inducer.

Materials

1. Sprague-Dawley rats (male)
2. 0.1 M citrate buffer
3. Glucometer and strips
4. Electrical signal pan balance
5. Oral feeding syringe
6. Syringes

Procedure

Sprague-Dawley rats weighing between 200-260 gm were brought. All rats were marked with picric acid and randomly divided into five groups, each comprising of six animals. Weight of individual rat was taken on electrical single pan balance and numbering was done to each rat.

Preparation of suspension of extract

Two formulations were prepared considering lower and higher doses in distilled water by sonication.

Preparation of solution of streptozotocin

Streptozotocin (65 mg/kg body weight of rat) in 0.1 M citrate buffer (pH 4.5).

Preparation of 0.1 M citrate buffer

Weigh accurately citric acid 10.5 gm and sodium citrate 14.7 gm. Mix it with 500 ml water. Make up volume to 1000 ml with distilled water. Adjust to pH 4.5tby sodium hydroxide.

Experimental design

Rats were divided into five groups, each group comprising of six animals. After overnight fasting (deprived of food for 16 hours had been allowed free access to water) diabetes was induced in group II,III,IV,V by intraperitoneal injection of STZ dissolved in O.1M sodium citrate buffer at pH 4.5, at a dose of 65mg/kg body weight.

Group I: Normal control received buffer solution for 28 days.

Group II: Diabetic control received only STZ for 28 days.

Group III: Diabetic rats received chloroform extract of *C.infortunatum* orally at a dose of 200 mg/kg body weight /day for 28 days.

Group IV: Diabetic rats received chloroform extract of *C.infortunatum* orally at a dose of 400 mg/kg body weight /day for 28 days.

Group V: Diabetic rats received Metformin 5 mg/kg body weight /day orally for 28 days.

Induction of experimental diabetes

- Diabetes was induced in rat by intraperitoneal (i.p.) injection of streptozotocin at a dose of 65 mg/kg body weight, dissolved in 0.1 M cold citrate buffer (pH 4.5). Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day post administration of streptozotocin[12].
- The animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycaemia.
- Control rats were injected with citrate buffer alone as placebo.
- The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on 3rd day of injection.
- The treatment was started on 4th day after STZ injection, considering it as 1st day of treatment. The treatment was continued for 28 days.
- Body weight of rats was taken on 0, 7th, 14th, 21st, 28th day of post treatment by electronic balance. Fasting blood glucose level of rats were taken on 0, 7th, 14th, 21st, 28th day of post treatment.

Oral glucose tolerance test (OGTT)

- The oral glucose tolerance test was performed in overnight fasted normal animals.
- Rats were divided into group of six animals each.
- The rat of all groups were given glucose (2 gm/kg body weight, orally) 30 min after administration of drug.
- Blood was withdrawn from tail- vein just prior to the drug administration (normal fasting) and at 0, 30, 60 and 120 min of glucose loading.
- Blood glucose level was measured immediately by using glucose oxidase - peroxidase reactive strips and a glucometer[13].

Biochemical parameters

The biochemical parameters like TG, total cholesterol, HDL, cholesterol, LDL cholesterol and VLDL were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit using Semi Autoanalyser. Blood glucose was measured using Accu check glucometer.

Estimation of Serum Triglycerides: (Enzymatic method)

Principle

Triglycerides + H20 Glycerol + Free fatty acids

Glycerol + ATP Glycerol-3-phosphate+ ATP

Glycerol-3- phosphate +O2 DHAP +H2O2

2H2O2 + 4 AAP Quinoneimine dye + 4H2O

The intensity of chromogen quinoneimine formed is proportional to the triglyceride concentration in the sample when measured at 510 nm.

Table-1: Procedure for TG estimation

Addition sequence	Blank (µl)	Standard (µl)	Sample (µl)
Working regent	1000	1000	1000
Distilled water	20	-	-
Standard	-	20	-
Sample	-	-	20

Mix well, incubate at 37 0c for 10 minutes. Measure absorbance of standard and sample against blank within one hour.

Estimation of HDL cholesterol: (PEG/CHOD-PAP method)

Principle: When the serum is reacted with polyethylene glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample for cholesterol using cholesterol (CHOD/PAP) reagent.

Contents

L1: Enzyme reagent 1
L2: Enzyme reagent 2
L3: Precipitating reagent
S: HDL cholesterol standard.

Working reagent

Pour 0.2 ml of L2 in to 0.8 ml of L1.

Precipitation of VLDL and LDL

0.1 ml of L3 and 0.1 ml of sample pipette in to a clean dry test tube. Mix well and incubate at room temperature for 5 min. Centrifuge at 2500-3000 rpm to obtain clear supernatant.

Table-2: Cholesterol assay: Pipette in to clean dry test tubes labeled as blank (B), standard(S) and test(T)

Addition sequence	B(ml)	S(ml)	T(ml)
Working Reagent	1.0	1.0	1.0
Distilled Water	0.05	-	-
HDL standard (S)	-	0.05	-
Supernatant*	-	-	0.05

Mix well and incubated at 37°c for 5 min.Measure the absorbance of S and T against B, with in 60 min.

*If only total cholesterol is to be determined use only 0.01 ml of distilled water/cholesterol standard/sample directly in cholesterol assay.

Calculation

$$\text{HDL cholesterol in mg/dl} = \frac{\text{Absorbance of T} \times 25 \times 2}{\text{Absorbance of S}}$$

Calculation of LDL Cholesterol (mg/dl) :(Freidewald's Formula)

= total cholesterol – triglycerides/5 – HDL cholesterol

Statistical Analysis

The experimental results were expressed as mean ± standard error of mean (SEM). Statistical testing methods included one way analysis of variance (ANOVA) followed by least significant differences test. p-values of less than 0.05 were considered to indicate statistical significance.

CONCLUSION

The pharmacological studies conducted on *Clerodendrum infortunatum* Gartn. indicate the immense potential of this plant in the treatment of conditions such as wounds, malaria, coughs, inflammatory, diabetes etc. *Clerodendrum infortunatum* Gartn. also exhibits antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, wound healing effect, anti-venom and anti-fertility activities.

However, the diverse pharmacological activities of *Clerodendrum infortunatum* Gartn. extracts and isolated phytochemical have been investigated in laboratory animals and the results obtained may not necessarily be portable to the situation in humans. While there are gaps in the studies conducted so far, which need to be bridged in order to exploit the full medicinal potential of *Clerodendrum infortunatum* Gartn. it is still clear that this plant with tremendous widespread use now and also with extraordinary potential for the future. Further research in phytochemicals development from *Clerodendrum infortunatum* Gartn. will help to analayse therapeutic efficacy of products. Efforts are now being made to investigate various therapeutic actions of *Clerodendrum infortunatum* Gartn. plant and their products using model systems.

ACKNOWLEDGEMENT

Auther is thankful to University Grant Commission, Westernal Regional Office, Ganeshkhind, Pune and Dr. George Augustine, Principal R. S. Bidkar College, Hinganghat Dist. Wardha for providing necessary facilities.

REFERENCES

[1] Barton, D.H.R., Cheung, H.T., Cross, A.D., Jackman, L.M. and M. Martin-Smith, M. 1961 Diterpenoid bitter principles. Part III. The constitution of clerodin. *J. Chem. Soc*5061 - 5073, DOI: 10.1039/JR9610005061.

[2] Bir, S.S. and Saggoo, M.I.H. 1979 *In* IOBP Chromosome number reports LXV. *Taxon* 28: 627-637.

[3] Hsiaosm J.Y,1995.. Botanical Bulletin of Academic Sinica Vol 36: P 247-251.

[4] Haines, H.H. 1925 The Botany of Bihar and Orissa. Vol. II. Bishen Singh and Mahendra Pal Singh, Dehradun, India.

[5]. Jirovetz, L., Buchbauer, G., Puschmann, C., Shafi, M.P. and Saidutty, A. 1999 Essential Oil Analysis of the Leaves and the Root Bark of the Plant *Clerodendrum infortunatum* used in Ayurvedic Medicine; *Herba Polonica* 45: 87-93.

[6] Kumar Manindra (2010) Medicinal properties of *Clerodendron infortunatum* Gaertn.

[7] Maisch, J.M. 1885 On some useful plants of the natural order of Verbenaceae. *American Journal of Pharmacy* 57, Botanical medicine monographs and sundry.

[8] Mitra, S. 1968 Cytotaxonomical studies on some members of Verbenaceae. *Proc 55th Indian Sci Congr* Part 3: 382.

[9]. Patil, R.P. 1958 Chromosome numbers of some dicotyledons. *Curr Sci* 27: 140-141.

[10] Rajakaruna, N., Harris, C.S. and Towers, G.H.N. 2002 Antimicrobial Activity of Plants Collected from Serpentine Outcrops in Sri Lanka. *Pharmaceutical Biology* 40 (3): 235–244.

[11] Rajurkar, B. M., April, 2010 Morphological Study and Medicinal Importance of *Clerodendrum infortunatum* Gaertn,(Verbaenaceae), Found in Tadoba National Park, India JPRHC 2(2):222-226

[12]. Roy, R, Pandey, VB, Singh, UP and Prithiviraj, B. 1996. Antifungal activity of the flavonoids from *Clerodendron infortunatum* roots. *Fitoterapia* 67:473-474.

[13] Sharma, A.K. and Mukhopadhayay, S. 1963 Cytotaxonomic investigation with the aid of an improved method on the family Verbenaceae with special reference to the lines of evolution. *Genetics* 58: 358-386.

[14] Shashtri, V.D. 1977 *Bhavprakash Nighantu* Motilal Banarsidas, New Delhi.

[15] Sinha, N.K., Pandey, V.B., Dasgupta, B., Higuchi, R. and Kawasaki, T. 1983 Acteoside from the flowers of *Clerodendron infortunatum Indian J. Chem* 22B: 97-98.

Source of support: Nil, Conflict of interest: None Declared