Pharmacognosy and biological activity of *Cordia rothii* Roem. & Schult. bark

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Received 31 August 2007; revised 29 June 2009

*Cordia rothii* Roem. & Schult. bark has reputation of being effective in heart ailments in Saurashtra region of Gujarat. The investigation was undertaken to study the microscopy, chemical constituents and pharmacological actions of *Cordia rothii* Roem. & Schult. bark. Phytochemical analysis of different bark extracts revealed the presence of alkaloids, glycoalkaloids, coumarins and inorganic salts. The aqueous extract of the bark was found to produce significant increase in force of contraction along with chronotropic effects on isolated rat heart preparations. The same extract did not show increase in urine output, however a significant increase in sodium as well as potassium excretion was noticed. Alcoholic extract was found to decrease glucose levels as compared to control. The study corroborates the cardiotonic activity attributed to *Cordia rothii* bark.

**Keywords:** Cardiotonic activity, *Cordia rothii*, Pharmacognosy, Coumarins, Glycoalkaloids

**IPC Int. CL:** A01K61/00, A61P9/00, A61P9/04, A61P9/06

*Cordia rothii* Roem. & Schult. (Boraginaceae) is a small tree growing mostly in tropical and temperate India, Sri Lanka and Abyssinia. In India, it is mostly found in Gujarat, Maharashtra, Rajasthan and Punjab. The bark of plant possess antidote activity, fruit pulp has astringent, antiarrhoeal & antiseptic activities and reduces burning sensation of urinary tract, root has abortifacient and anti-inflammatory activities, while the whole plant has antidiabetic & antileprotic activities. In Ayurveda, the plant is considered as a source of *Laghusleshmataka*, a substitute of *Sleshmataka* (*Cordia dichotoma* Forst.), the bark of which is used in dyspepsia and fevers. The bark is used in heart ailments, and its efficacy was found to be better than other Ayurvedic cardiac drugs, like *Arjuna* (*Terminalia arjuna* W. & A.) bark and *Javvar* (*Pterocarpus marsupium* Roxb.) wood. β-sitosterol (from stem and leaf), D-galactose, D-fructose, D-xylose, L-rhamnose and D-galactouronic acid (from fruit mucilage), hydrocarbon and n-hexacosanol (from leaf) have been reported from the plant. Phytochemical investigations of other species of *Cordia* have been reported, majority of them are found to be exotics. Morphological, microscopical, and pharmacological studies of the bark have not been reported. The contribution besides providing these data corroborates the claim.

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**Methodology**  
Young and old stem barks of *Cordia rothii* Roem. & Schult. bark were collected from the campus. The identity of the tree was confirmed by literature. Fresh bark was used for the histochemical studies and dried powdered (40#) was used for extraction, isolation of the secondary metabolites and for pharmacological studies. Morphological characters of both young and old barks were studied. Free hand sections a few barks pieces soaked in a mixture of Formalin-Aceto-Alcohol in ratio of 9:5:5 for 12 hrs were taken. The sections were observed after clearing with chloral hydrate and staining with various reagents for histochemical studies. Lignified elements were isolated by Schultz maceration technique. The drawings were made and the dimensions of the cellular elements were measured. Powdered bark was subjected to the various chemical tests to detect the presence of sterols, alkaloids, glycosides (cardiac, anthraquinone, flavonoid, steroidal and coumarins), etc. Powdered bark (100 gm) was refluxed for 2 hrs, thrice using petroleum ether (60-80°C). The solvent was distilled off leaving behind a dry residue. It was weighed and dissolved in 25 ml chloroform and the solution was tested for the presence of sterols by Libermann-Burchardt and Salkowski test. The defatted powder was basified with ammonia solution, dried at 60°C and was extracted thrice by refluxing for 2 hrs by
using 1.5 L of chloroform each time. The chloroform extract was concentrated to 50 ml and extracted with 5% HCl (30 ml × 5 times). Combined acid extract was made alkaline to pH 10 by using ammonia solution and was further re-extracted successively with chloroform (30 ml × 4 times). After removing its moisture with sodium sulphate, it was concentrated to dryness under reduced pressure, weighed and subjected to alkaloid tests and TLC studies using silica gel G as a stationary phase and thymosulphuric acid as a spray reagent. The precipitates were dissolved in 50 ml water, ammoniated to 150 ml under reduced pressure and cooling it to physical, organoleptic and group detection tests (anions and cations)\(^\text{17}\). The marc left after the isolation of the alkaloids was refluxed for 2 hrs, using 1.5 L of ethanol thrice. The ethanolic extract was set aside for 15 hrs at 5°C, white crystals settled at the bottom were separated by concentrating the mother liquour to 150 ml under reduced pressure and cooling it to physical, organoleptic and group detection tests (anions and cations)\(^\text{17}\).

The ethanolic mother liquor left after removal of white crystals was treated with 300 ml of acetone. Buff coloured precipitates that formed were allowed to settle at 5°C for 15 hrs. The precipitates were separated, dried, weighed and subjected to various tests like Molisch’s, Borntrager’s, Keller-Killiani’s, Libermann-Burchardt, Dragendorff’s, Shinoda’s\(^\text{15,18}\). TLC separation was carried out using silica gel G as a stationary phase, acetic acid: ethanol (20:60) as a mobile phase and Dragendorff’s reagent as a spray agent\(^\text{16}\). The marc left after the isolation of the alkaloids was refluxed for 2 hrs, using 1.5 L of ethanol thrice. The ethanolic extract was set aside for 15 hrs at 5°C, white crystals settled at the bottom were separated by concentrating the mother liquor to 150 ml under reduced pressure and cooling it to physical, organoleptic and group detection tests (anions and cations)\(^\text{17}\).

Pharmacological screening

Powdered bark (100 gm) was extracted thrice, each time refluxing it with 400 ml of water for 2 hrs. The aqueous extract was concentrated to dryness under reduced pressure. A portion of it was dissolved in water to obtain the concentration of 100 µg extract/ml. The cardiac actions (heart rate and its force of contraction) were studied by administering the extract of the drug at the dose of 10, 20, 40, and 80 µg, respectively in the isolated hearts of the rats by Langendorff’s method\(^\text{21,22}\). From the above dried aqueous extract, a portion was suspended in water with the help of 1% Acacia in water to get the concentration of 25 mg extract/ml. The diuretic activity was studied\(^\text{21,22}\). Albino rats (Wistar strain) of either sex weighing 180-220 gm were used for the experiment. The animals were maintained in the animal house and were fed on Lipton’s Gold Mohr feed. They were divided into two groups, control and treated, each having 5 rats. The animals were fasted overnight and allowed free access to drinking water before the experiment. Distilled water in the dose of 5 ml/100 gm body weight was administered orally, twice (before and 60 minute after drug administration). The extract was administered at the dose of 250 mg/kg body weight orally to the treated group and equivalent quantity of water with 1% Acacia to the control group. All the rats were housed in individual metabolic cages and urine was collected for a period of 5 hrs. Urine volume output was noted and sodium and potassium content was determined by flame photometry.

Powdered bark (100 gm) was extracted thrice, each time refluxing it with 400 ml of ethanol for 2 hrs. The alcoholic extract was concentrated to dryness under reduced pressure. The dried residue was suspended in water using 1% Acacia to obtain the concentration of 25 mg extraction/ml. Dextrostix and Glucometer were used for estimating the blood sugar level\(^\text{23}\). Adult albino rats of either sex (Wistar strain), weighing 175-230 gm were divided into control and treated groups, each containing 5 animals. The rats were fasted for 24 hrs before starting the experiment. Fasting blood sugar level was measured by taking blood from the tail vein on the dextrostix. Thereafter, orally, glucose
solution (750 mg/kg body weight) was immediately administered to all rats and after an hour their sugar level was estimated. The treated and control group of animals then, were orally administered with 250 mg/kg body weight of the extract and water: *Acacia* (100:1) solution, respectively and their blood sugar level was re-estimated again for 3 hrs at interval of one hour.

**Results and discussion**

The dried bark is curved to channeled in shape; young bark, 10-20 cm in length, 12-20 mm in width and 1-2 mm in thickness with smooth greenish brown outer surface traversed by transversely elongated whitish lenticels. Older barks are thicker (3-5 mm) and with very rough external surface due to presence of rhytidoma. Inner surface is longitudinally striated, whitish in colour when fresh and turning to brown on drying. The fracture is splintery in cork and cortex, and laminated in the phloem. The diagrammatic transverse section shows narrow cork, cortex and pericyclic zone containing stone cells as well as groups of fibres and wide phloem with multiseriate medullary rays and tangentially running bands of fibres. Odour is slight but characteristic and the taste is bland. In detailed transverse section, young bark is covered externally with distorted epidermis and cuticle which gets peeled off in older bark, consisting of 5-6 rows of tabular tangentially elongated suberised cells, interrupted at places by groups of oval to circular parenchymatous cells of the lenticels. One to two layers of thin walled, tangentially elongated, yellowish brown, cork cambium cells lie underneath the cork. Cortex is narrow, 5-6 layered, parenchymatous and is traversed by small and big sized stone cells isolated or in groups of 2-3. Smaller stone cells are few in number, thick walled and 10-16 µ in length and 9-13 µ in width. Pericyle is characterized by the presence of stone cells and groups of thick walled lignified fibres. Stone cells are 10-15 µ in length and 6-10 µ in width and are located at the peripheral region of the pericycle. Phloem is characterized with the tangentially running bands of thick walled lignified fibres alternating with sieve tubes, parenchyma and companion cells. The primary phloem is very wide and forms the major bulk of the bark. It is traversed by obliterated tissue and multiseriate medullary rays, which get broader or wedge-shaped towards peripheral region and contain groups of pitted, lignified, thin walled stone cells measuring 25-39 µ in length and 18-21 µ in width. The secondary medullary rays uni to triseriate and the cells are radially elongated. Microsphenoidal and prismatic crystals of calcium oxalate traverses throughout the parenchymatous cells of the section.

Chloroform extract gave positive Libermann-Burchardt and Salkowski tests indicating the presence of sterols. 0.0313% w/w, was the yield of the petroleum ether extract of the bark. Alkaloid yield was 0.0116% w/w was the yield of the defatted basified powder of the bark. When tested with Dragendorff’s reagent it gave positive test, and the resolved spots on TLC (Rf- 0.632 and 0.688) also gave orange colour, confirming the presence of alkaloids in the bark. The yield of the white prismatic crystals obtained from the alcoholic extract of the powder (devoid of sterols and alkaloids) was 0.62% w/w. It did not burn or melted on ignition and when tested chemically proved to be the crystals of potassium chloride. The yield of the buff coloured precipitate obtained on addition of acetone in the alcoholic extract (devoid of sterols, alkaloids and salts) of the bark was 0.064% w/w. It did not respond to any other test except Molisch, Libermann-Buchardt and Dragendorff’s tests, indicating the possibility of its glycoalkaloidal nature. On TLC it resolved at Rf 0.533 and gave violet colour with Libermann-Buchardt spry reagent. TLC studies, IR spectral analysis and decomposition at 158-173°C of the sugar moiety melted at 208-212°C, resolved at 0.458 Rf and gave orange colour with Dragendorff’s reagent. Its IR spectra showed peaks at 3440, 3280, 2900, 1670, 1260, 1060, 860, 800, 620 cm⁻¹ indicating its nitrogen containing steroidal structure. Thus, it was identified as a glycoalkaloidal made up of steroidal aglycone moiety and sucrose moiety. A drop of alcohol: acetone solution (devoid of sterols, alkaloids, salts and glycoalkaloids) on Whatman paper, when exposed to ammonia showed a dark blue fluorescence under UV light. Its TLC separation showed nine different coloured spots under UV light, as brilliant blue (Rf 0.06), brown (Rf 0.104), sky blue (Rf 0.14), olive green (Rf 0.38), brilliant blue (Rf 0.48), yellowish green (Rf 0.61), grayish blue (Rf 0.64), reddish brown (Rf 0.73) and yellowish green (Rf 0.80). With iodine-potassium-iodide as spray reagent, only two spots out of these, developed reddish brick red (Rf 0.61) and violet brick red (Rf 0.80) colour indicating them to be of coumarins.

The bark aqueous extract was found to produce significant positive inotropic and chronotropic effects
on rat’s heart. The cardiac stimulating action was observed only after of 10, 20 and 40 µg of the extract; 80 µg of the extract produced arrhythmia in the heart. The average chronotropic effect (38.7 ± 2.2) was less than the increase in the force of contraction in heart. This suggests that Cordia rothii possess a cardiotonic activity. Cardiotoxic drugs like digitalis are known to possess diuretic activity; in the study, any increase in urine volume was not found (Table 1). The absence of diuretic activity probably is due to the fact that the experiments were carried out on normal animals and not on rats with degenerated hearts. However, increasing sodium and potassium in urine excretion can be considered as an additional benefit in a drug having cardiotonic activity. Increasing potassium excretion could be due to high potassium content of the plant but increasing sodium content revealed a natriuretic activity of the plant. Cardiac failure is often found to be reported in diabetic patients; hence a cardiotonic drug with antidiabetic activity like Cordia rothii may be all the more beneficial in such patients. The alcoholic extract of Cordia rothii bark was found to possess significant hypoglycaemic activity (Fig. 5).

Conclusion

Bark of Cordia rothii Roem. & Schult. contains alkaloids, glycoalkaloids, coumarins and salts of potassium chloride. It showed cardiotonic and antidiabetic activities. The activities need further evaluation like its comparison with standard drug, clinical studies, etc. The detection of the compounds responsible for these activities is in progress.

References