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Extraction and isolation of clerodane as a bioactive molecule from *Tragia ramosa*

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Abstract
Medicinal plants have been commendable source for as an alternative remedy for treating numerous diseases, because of the plant having analgesic activity, antibacterial activity, antioxidant activity, insecticidal activity & cytotoxicity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products interest particularly in *Tragia ramosa* plants has grown throughout the Maharashtra. The focus of this paper is on the analytical methodologies, which include extraction, isolation & characterization of active ingredients from *Tragia ramosa* plant, As extraction is the most important steps in the analysis of bioactive molecule present in plant extracts. The analysis of bioactive compounds present in the plant extracts was characterized by Fourier transform Infrared (FTIR) spectral & ^{1}H NMR method.

Keywords: Extraction, isolation, clerodane, bioactive molecule, *Tragia ramosa*

Introduction
Tragia ramosa is a species of flowering plant in the euphorb family known by the common names branched *noseburn* and *desert tragia* [1]. It is native to the southern Great Plains, South Central, and Southwestern United States and Northern Mexico [2]. It grows in scrub, woodland, and other desert and plateau habitat. *Tragia ramosa* is a perennial herb growing mostly erect, measuring 10 to 30 centimeters in maximum height. It is covered in long, rough stinging hairs. The leaves have lance-shaped or oval blades with toothed edges which are borne on petioles. The plant is monoecious. Its inflorescence contains a few male flowers and usually one female flower. The flowers lack petals but have green sepals. The female flower yields a small capsule.

Fig 1: *Tragia ramosa* Plant

This perennial herb is small and delicate - looking, but be careful because the whole plant is covered with stinging hairs. Look for the slender stems which turn brown with age; the leaves longer than they are wide, with toothed edges; and if you catch it at the right time, one or two seed pods attached to each stem, usually an inch or two below the stem tip. The seed pods have 3 lobes and explosively split open to release the seeds. Difficult to distinguish from *T. nepetefolia*, which has more flowers per raceme (6-many), a shorter papillose style, and 3 stamens. *T. nepetefolia* is also found at lower altitudes, down to 2,500 Ft. (762 m).
Study on medicinal plants is becoming an important area of study. Micro-organisms are becoming resistant to drugs used to kill them, hence the need for alternative drugs to treat them. Many scientists have turned to plants to obtain these compounds. The use of medicinal plants to treat various types of diseases was very important in ancient days, since there were no commercial medicines by then.

The introduction of industrially produced drugs has however led many people to turn from plants to use these synthesized products. However the trend is changing with many people turning to plants for treatment. Some important bioactive molecules present in green plant are [3-5],

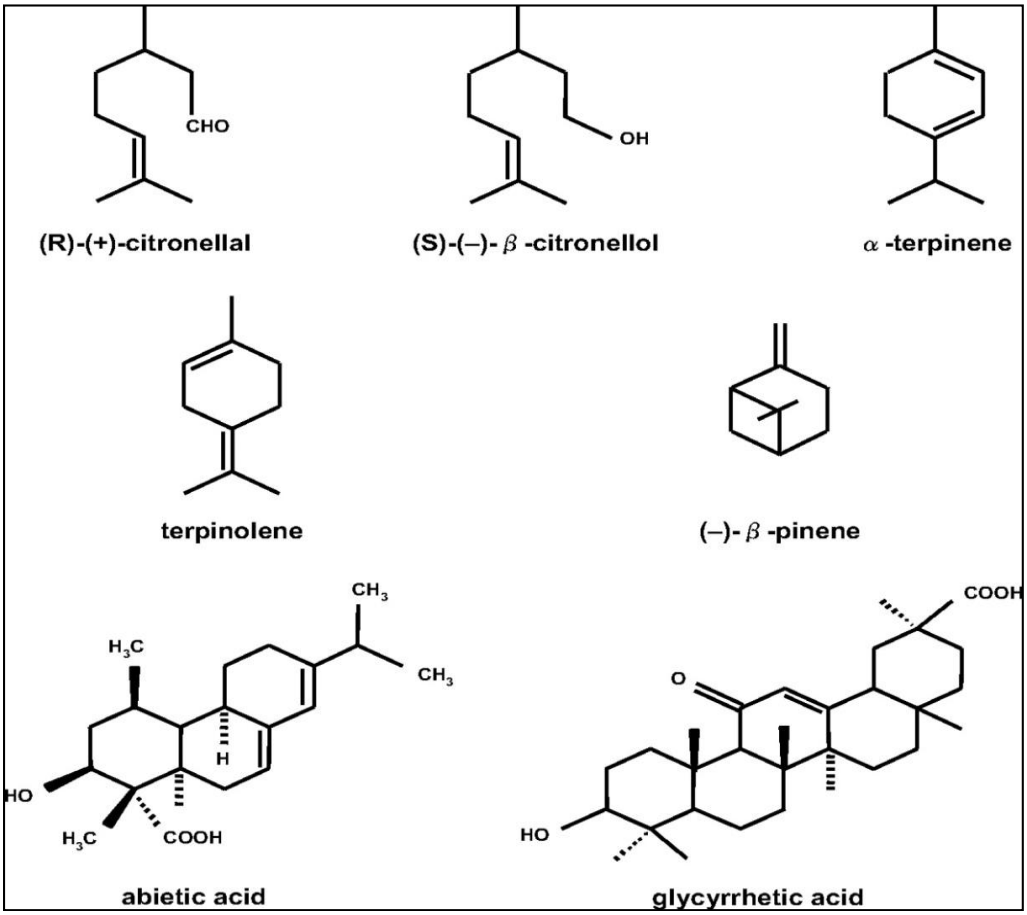


Fig 2: Bioactive molecules present in green plant

Material and Methods

Plant Materials

Leaves of *Tragia ramosa* plants (*Branched nosburn*) from the family Euphorbiaceae were collected from our college campus, Murum, during the month of October The plants were identified and authenticated by taxonomist, Dr. Bajgire R.S. Department of Botany, S.M.P. College, Murum and Dr. Nilesh Pawar, Department of Botany, New College, Kolhapur.

The collected leaves were washed with distilled water, and shade dried till it is crisp (approximately 15 days). The dried leaves were grinded until it converts into powdered form. The powder was stored in freezers at 4 ℃ for further use.

Fig 3: Sample for extraction

Experimental Work

The powdered material placed inside a thimble made from thick filter paper, which is loaded into the main chamber of Soxhlet extractor. The Soxhlet extractor is placed into a flask containing the ethanol. The Soxhlet is then equipped with a condenser [6-11].

Fig 4: Soxhlet *extractor*

The ethanol is heated to reflux. The ethanol vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm ethanol. Some of the desired compound will then dissolve in the warm ethanol. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the ethanol running back down to the distillation flask. This cycle is allowed to repeat for 4 hours.

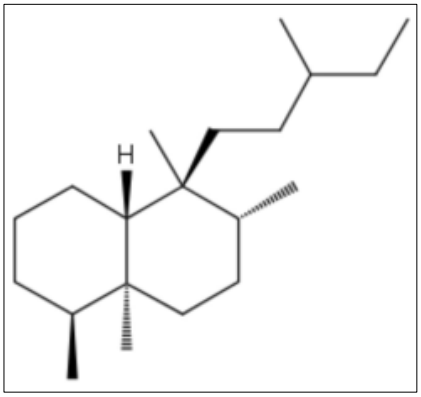
During each cycle, a portion of the non-volatile compound dissolves in the ethanol. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm ethanol being passed through the sample, just one batch of ethanol is recycled.

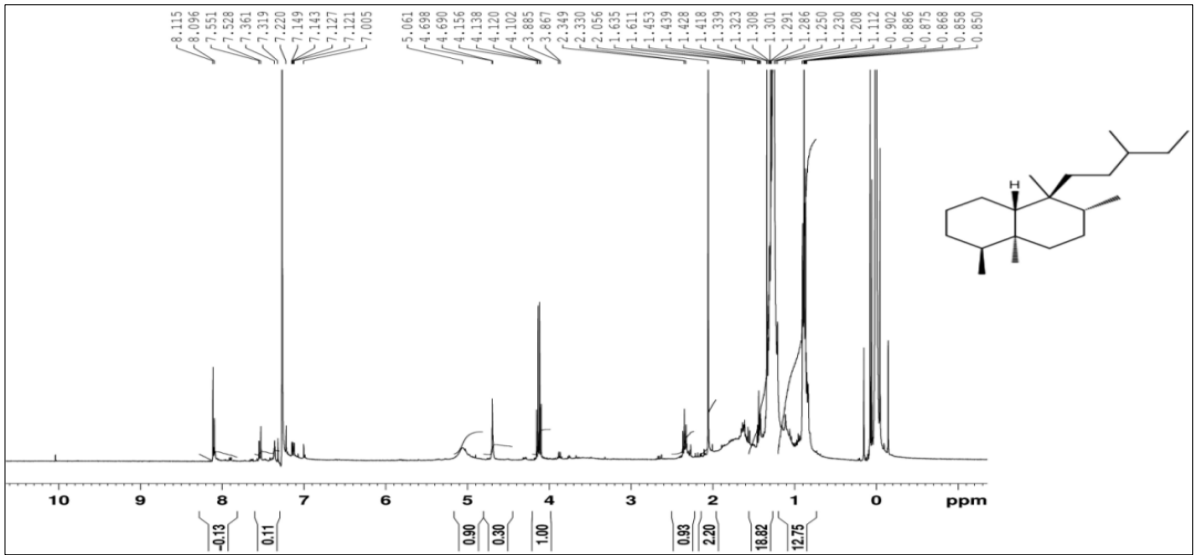
After extraction the ethanol is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

After getting desired product, the purity of the compound was checked by TLC. For further analysis the sample are collected for IR and 1H NMR.

Result & Discussion

The extract obtained were dark brown in colour. From TLC Observation it concludes that, the product obtained is highly pure because it shows only one single spot on TLC Plate. From literature survey and our opinion we conclude that *Tragia Ramosa* contain Terpenoids as major Organic compound. Further study of IR and 1H NMR spectral analysis, no peak of aromatic ring at the region 3140cm^{-1}. It indicates the absence of aromatic ring compounds. From IR, the peak at 2944cm-1, indicate aliphatic -C-H stretching frequency. From ^{1}H NMR spectral data it conclude that the absence of aromatic hydrogen in the region δ 7 - 8 ppm. It conclude that total 37-aliphatic hydrogen are present in our product. By literature survey, *Tragia Ramosa* plant leaves extracted by soxhlet extractor by ethanol as a solvent only terpenoid Clerodane ($C_{20}H_{38}$) was a major product. It conclude that *Tragia Ramosa* contain Clerodane is a major organic bioactive molecule.

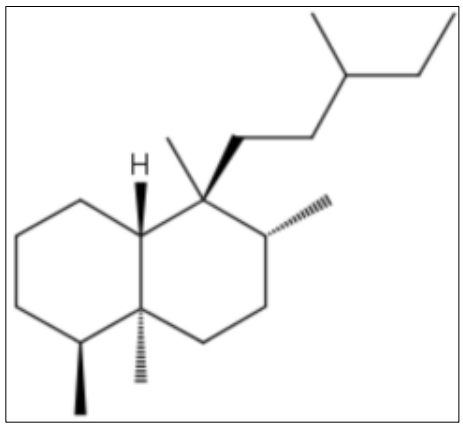




^{1}H NMR spectra of isolated product

Conclusion

We have isolated single Clerodane from *Tragia Ramosa* and confirmed by IR & ^{1}H NMR spectral data.



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