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Short Communication

# PHYTOCHEMIMCAL ANALYSIS OF WRIGHTIA TINCTORIA BARK EXTRACT IN WATER USING GC-MS

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**ABSTRACT**

**Objective:** In the present study, phytochemical constituents of crude water extract of *Wrightia tinctoria* (WT) bark is done using GC-MS technique. *Wrightia tinctoria* is an important medicinal herb being used in tribal areas of Chhattisgarh since long but chemical constituents of its bark responsible for the activities are still not studied in depth.

**Methods**: Dried bark powder was successively extracted with petroleum ether, ethyl acetate and methanol using soxhlet apparatus and lastly material was dissolved in distilled water for 10 hrs for extraction. Water extract was selected for the further analysis using Agilent 7890A GC with 5975MS.

**Results**: As per the GC-MS analysis, twelve different compounds namely benzene 1, 2, 4, 5-tetramethyl (2.85%), benzene 1, 2, 3, 5-tetramethyl (1.16%), 1-decanol, 2, 2-dimethyl (4.38%), phenol 2, 4-bis (1, 1-dimethyl ethyl) (7.78%), heptadecane (3.60%), 3-hexadecanol (3.30%), i-propyl tetradecanol (3.64%), benzo (h) quinoline (3.66%), n-hexadecanoic acid (6.54%), octadecanoic acid methyl ester (0.81%), phytane (1.95%) and pentadecane (2.25%) were characterized. Analysis and identification of presence of the compound in these extracts were done using the database of NIST library.

**Conclusion:** Study confirms the presence of biologically active phyto-constituent in water extract of *Wrightia tinctoria* bark those may be the key chemical of natural origin in new drugs designing against major disease those are being treated in tribal areas using this plant bark. Further confirmation of *in vitro* bioactivity using cell line culture is required which is planned as the future prospect of current study.

**Keywords:** *Wrightia tinctoria,* Phytochemical, GC-MS analysis, *Apocyanaceae.*

Natural medicines are being used worldwide in the variety of ways for clinical health care and as home remedies. Natural medicine may be derived from microorganism, insects, animals, sponges, spiders or reptile but the plants are the potential and major source of natural medicines since ancient time till date. In some developing countries, society relies profoundly on traditional health practitioners and plants to meet their vital health care requirements. In many developed countries herbal medicines are gaining pace as alternative and complementary therapies [1].

Plants are being investigated extensively for their pharmacological purpose as the source material of major modern drugs. Crude extracts of different parts of medicinal plants were being used to treat different type of infectious diseases in Ayurvedic medicine system but recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance in pure form from higher plants for specific diseases [2-4].

Tribal knowledge about the traditional medicines are the wealth of India and abroad. Chhattisgarh state of India is also a major tribal state with treasure of traditional knowledge for use of plant product in order to cure various diseases. *Wrightia tinctoria* is one of the tribal medicinal plants in Chhattisgarh which is being used to treat the variety of the disease but still knowledge about chemical constituents in whole of this plant is not up dated [4].

*Wrightia tinctoria* (Roxb) R. Br. belong to family *Apocynaceae* [5] is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India[6]. Some important photochemical isolated are wrightial, cycloartenone, cycloeucalenol [7], indigotin, indirubin, tryptanthrin, isatin, rutin[8], β-sitosterol, β-amyrin, wrigatiadione [9] reported in seeds, leaves and bark of this plant. WT bark is extensively used as important medicine in tribal areas [10-16] but detailed chemical profiling is still a missing link in our knowledge. In the present study, investigations were carried out to determine the possible phytochemicals component in crude water extract of WT bark by GC-MS analysis.

Bark of WT was collected in the month of September from the tribal areas of Ambikapur (Wadrafnagar, Madhna GPS: Latitude: 23$^0$47'05.8"N; Longitude: 83$^0$06'20.8"E) Chhattisgarh India. Plant was taxonomically identified by Professor Dr. K. P. Sahu, Botany Department, Govt. Model Science College Jabalpur. Dried bark powder was successively extracted with petroleum ether, ethyl acetate and methanol using soxhlet apparatus and finally after extraction with above solvents material, from the soxhlet was taken out and was dissolved in distilled water for 10 hrs for hydro extraction. Water extract was purified by thrice filtration with Whatman filter paper#1 and subsequent distillation along with charcoal treatment and stored in refrigerator till use. Only water extract was selected for the final analysis and confirmation of chemical composition because in separate study other extracts along with water extract in sequential extraction showed differential response against *Mycobacterium tuberculosis* [data under publication].

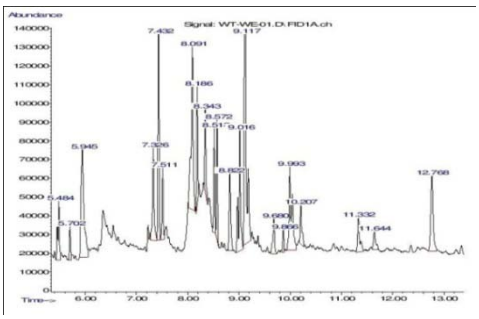


**Fig. 1: GC spectrum of crude WT bark extract in $H_2O$ Showing major compounds peak**

For the analysis of the extracts, Agilent 5975C TDA series gas chromatography/mass spectroscopy with selective detector system was used which offer high performance and flexibility with many options. Crude water extract (1 µl) of WT bark was used in GC-MS analysis with Agilent (5975C MS) 5% poly siloxane column of 30×250 µm×0.25 µm size. Oven temperature was programmed as: Isothermal temperature was 5ºC/min and held for 1.75 min then increased to 275 °C at the rate of 8ºC/min and kept constant for 5 min. The run time was 25 min. Ionization of sample components were performed on EI mode (70eV).

Interpretation of GC-MS spectrum was done using the database of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Name, molecular weight and structure of compounds of the water extract were ascertained.

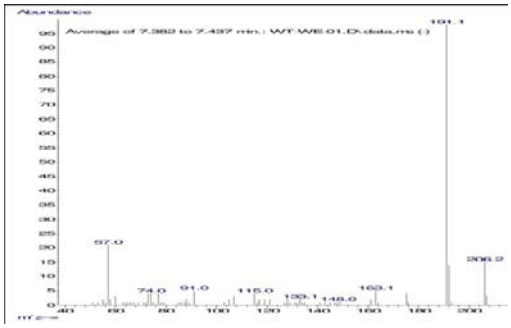

**Fig. 2: Mass Spectrum of Phenol 2, 4-bis (1, 1-dimethyl ethyl)**

Results showed the presence of benzene 1, 2, 4, 5-tetramethyl (2.85%), benzene 1, 2, 3, 5-tetramethyl (1.16%), 1-decanol, 2, 2-dimethyl (4.38%), phenol 2, 4-bis (1, 1-dimethyl ethyl) (7.78%), heptadecane (3.60%), 3-hexadecanol (3.30%), i-propyl tetradecanol (3.64%), benzo (h) quinoline (3.66%), n-hexadecanoic acid (6.54%), octadecanoic acid methyl ester (0.81%), phytane (1.95%), pentadecane (2.25%).

List of 12 compounds detected in crude water extract of WT bark with their retention indices, percentage composition, chemical structure and activities are given in table-1 and fig. 1-3.

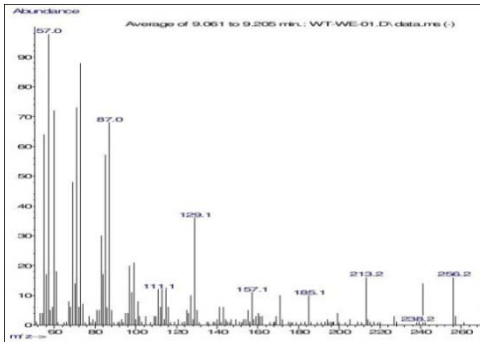

**Fig. 3: Mass Spectrum of n- Hexadecanoic acid**

Among the identified phyto chemicals, n-hexadecanoic acid and Phenol 2, 4-bis (1, 1-dimethyl ethyl) has a role in antioxidant effect [17-18] present in maximum percentage. Phenol 2, 4-bis (1, 1-dimethyl ethyl) have good antibacterial activity [19]. Previously no results are discussed in available literature about the chemical composition of WT bark but a same compound, n-hexadecanoic acid was found already reported in leaves of WT plant. 3-O-Methyl-d-glucose (51.44%) was reported as major compound along with 21 minor compounds in ethanolic extract of WT leaves with GC-MS analysis [20].

**Table 1: Shows compounds identified in crude waster extract of WT using GC/MS.**

| S. No. | RT(in minutes) | Name of the isolated compound | Molecular formula | MW (amu) | Peak % area |
|---|---|---|---|---|---|
| 1 | 5.48 | Benzene 1, 2, 4, 5-tetramethyl | $C_{10}H_{14}$ | 134 | 2.85 |
| 2 | 5.70 | Benzene 1, 2, 3, 5-tetramethyl | $C_{10}H_{14}$ | 134 | 1.16 |
| 3 | 7.32 | 1-decanol 2, 2-dimethyl | $C_{12}H_{26}O$ | 186 | 4.38 |
| 4 | 7.43 | Phenol 2, 4-bis (1, 1-dimethyl ethyl) | $C_{14}H_{22}O$ | 206 | 7.78 |
| 5 | 8.18 | Heptadecane | $C_{17}H_{36}$ | 240 | 3.60 |
| 6 | 8.51 | 3-hexadecanol | $C_{16}H_{34}O$ | 242 | 3.30 |
| 7 | 8.57 | i-Propyl tetradecanol | $C_{17}H_{34}O_2$ | 270 | 3.64 |
| 8 | 8.82 | Benzo (h) quinoline | $C_{13}H_9N$ | 179 | 3.66 |
| 9 | 9.01 | n-hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 6.54 |
| 10 | 9.86 | Octadecanoic acid methyl ester | $C_{19}H_{38}O_2$ | 298 | 0.81 |
| 11 | 10.20 | Phytane | $C_{20}H_{42}$ | 282 | 1.95 |
| 12 | 11.33 | Pentadecane | $C_{15}H_{32}$ | 212 | 2.25 |

Hexadecanoic acid and 1, 5-methyl methyl ester (58.30%) were reported as major compound along with minor 10 compounds in ethanolic extract of WT flowers by GC-MS analysis [22]. No similar compound was found in crude water extract of WT bark as reported in flower. In this study, presences of twelve components resolved by GC-MS are being reported. This study provided with the new knowledge for the upcoming research in recent future.

## CONCLUSION

In the present study twelve chemical constituents have identified from the water extract of WT bark by GC-MS analysis those are not reported in bark of this plant in already published literate. Some of the peaks could not be identified which may be the next step for further investigation along with the detailed bioactivity of compounds identified.

## ACKNOWLEDGEMENT

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## ABBREVIATION

WT - *Wrightia tinctoria;* WE - Water Extract; NIST - National Institute of Standards and Technology; TDA - Toluene-diamine; EI - Electron Ionization; PCI - Positive Chemical Ionization; NCI - Negative Chemical Ionization; RT - Retention Time; MF - Molecular Formula; MW -molecular Weight; Amu - Atomic mass unit; MS - Mass spectroscopy/mass spectrum.

## CONFLICT OF INTERESTS

Declared None

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