In- Vitro Pharmacological Investigation And GC-MS Analysis Of Rhodiola Rosea Root Against Bacterial And Helminthiasis Infections

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Abstract:

The present study's objective was to identify the phytoconstituents present in Chloroform extract of *Rhodiola rosea* (*R. rosea*) with a help of Gas chromatography-Mass Spectrometry (GC-MS). The extract was subjected for the antibacterial (*S. aureus* and *Pseudomonas aeruginosa*) and anthelmintic (adult Indian earthworms-*Pheretima posthuma*) *in-vitro* activities followed by the validation of the same by subjecting the bioactive compounds obtained in GC-MS analysis to Sixteen compounds with molecular formula $C_{24}H_{46}O_4$, $C_{16}H_{44}O_3S$, $C_{35}H_{70}$,$C_{21}H_{44}O_2$,$C_{24}H_{38}O_4$, $C_{21}H_{24}O_7$, $C_{12}H_{23}O_2N$, $C_{30}H_{50}$, $C_{35}H_{70}$, $C_{21}H_{44}O_3S$, $C_{26}H_{52}O_2$, $C_{26}H_{44}O_5$, $C_{20}H_{40}$ and $C_{16}H_{33}Cl$ were identified in GCMS analysis. Different extract concentrations viz 25, 50, 75, and 100 mg/ml and 25, 50, 75, and 100 µg/ml for anthelmintic activity and anti-bacterial activity respectively. The extract exhibited dose-dependent *in-vitro* anthelmintic (50,75 and 100 mg/ml) and antibacterial activity. The minimum lethal amount of extract concentration for anthelmintic activity was found to be 75and100 µg/ml. At this concentration, *Pheretima posthuma* died in 32.15 ± 3.30 min while with a standard dose of albendazole, the lethal time was found to be 22.75 ± 1.63 min. Finally concluded that the identified bioactive compounds from *R. rosea* have the potential anthelmintic and antibacterial activity as justified by *in-vitro* biological evaluations studies.

Keywords: Rhodiola rosea, *GC-MS,* S. aureus and Pseudomonas aeruginosa
DOI Number: 10.14704/nq.2022.20.9.NQ440051 **Neuro Quantology 2022; 20(9):487-491**

INTRODUCTION:

The genus *Rhodiola* of family Crassulaceae nearly around 200 species present, in which almost 20, comprising *Rhodiola rosea*, *Rhodiola alterna, Rhodiola brevipetiolata, Rhodiola crenulata, Rhodiola kirilowi, Rhodiola quadrifida, Rhodiola sachalinensis*, and *Rhodiola sacra*, are used as ethno-medicines in Asia[1] .*Rhodiola rosea* plants mainly available in the region of Himalayan belt, Tibet, China, and Mongolia, were as also cultivated in Europe and North America, *Rhodiola rosea* are available dietary supplements and juice on the market.[2-3] widely known as "golden root" or "roseroot." The pharmacological effects of *Rhodiola rosea* are adaptogenic or anti-stress, hepatoprotective effects and immunomodulatory, antiviral, anti-inflammatory, and antibacterialactivities,[1,4] *Rhodiola rosea* contain secondary metabolites Such as polyphenols, flavonoids, proanthocyanidines, tyrosol, and cinnamyl alcohol, glycolsides, organic acids, essential oils, sugars, fats, alcohols, and proteins[4]. The polyphenol includes Rosavin, cinnamyl alcohol, salidroside and tyrosol major components of *Rhodiola rosea* [5-6]. The aim of present study is to identification of bio active compounds in chloroform extract *R. rosea* on application of Gas chromatography-mass spectrometry (GC-MS) there *in-vitro* antibacterial and anthelmintic activity of *R. rosea*.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL:

Dried roots of *Rhdiola rosea* (Golden root) family Crassulaceae were procured from local vendor, Seremban, negeri Sembilan, Malaysia.
Roots are authenticated by Dr. Long chiau Ming, Associated Professor, Deputy Dean faculty of Pharmacy, Quest international university Perak. (QUIP-RR/02/2019).

PREPARATION OF EXTRACTION

The roots material was powdered using mixer grinder and passed through sieve no 85. About The dried 150gm powder was subjected to

soxhlets apparatus extraction using methanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator.

CULTURE MEDIA BACTERIA

S. aureus ATT 25923 and *Pseudomonas aeruginosa* ATCC 27853 microorganisms provided by Owaisi Hospital and Research Center, department of microbiology, Hyderabad, Mueller-Hinton agar (Himedia, Lot 0000333943, Code M173) NaCl, Beef Extract procured by Himedia Pvt. Ltd., India. *P. aeruginosa*

GC-MS ANALYSIS:

Chromatographic data were obtained using Clarus 680 GC. A fused silica column packed with Elite-5MS (containing, 5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df), separation of components was carried out using Helium gas with a constant flow rate of 1ml/minute. Whereas the injector temperature was maintained at 260°C throughout the chromatographic. The volume of the extract injected into the instrument was 1 µL. The oven temperature was initially maintained at 60 °C for two minutes, followed by 300 °C at the rate of 10 °C min−1 for 6 minutes.

Meanwhile, for the mass detector, transfer line temperature and ion source temperature was maintained at 240° C. Ionization mode was set with an electron impact of 70 eV with 0.2 seconds, scan time and 0.1-sec scan interval. Fragmentation was observed between 40 to 600 Da. The resultant spectra were compared with the reference spectrum using GC-MS NIST (2008) library.

PREPARATION OF EXTRACTS:

The *Rhdiola rosea* chloroform extract different concentration were prepared 25, 50, 75, and 100 mg/ml for anthelmintic activity and 25, 50, 75, and 100 µg/ml for anti-bacterial activity. Albendazole 20mg/kg bw as standard. In each Group contain 3-4 *Pheretima posthuma* earthworms approximately equal size are individually placed in each petridish fill with10-15 ml normal saline solution and desired concentration of drug and extracts.

ANTIBACTERIAL TESTING BY AGAR WELL DIFFUSION METHOD

Assessment of antibacterial activity *R.rosea* extracts was determined by agar well diffusion method described[7]. Inoculums of *S. aureus* and *Pseudomonas aeruginosa* bacterial strains were plated using sterile swabs into Petri dishes containing approximately 25 ml of Nutrient agar media, where 6 mm wells were made and filled with different concentration 25,50,75 and 100µl/ml of extracts. The Petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples and then, incubated at 37±1°C for 24 h. Further antibacterial activity of the above samples was determined by measuring the zone of inhibition millimeters (mm) according to the standard clinical parameters.

ANTHELMINTIC ACTIVITY:

The *in-vitro* anthelmintic study was done as per the protocol described by. [8-9]

Group 1: Normal Control Group using normal saline 0.5% w/v
Group 2: Standard Group Albendazole 20 mg/ml kg bw
Group 3: *R. rosea* Chloroform extract 25 mg/ml
Group 4: *R. rosea* Chloroform extract 50 mg/ml
Group 5: *R. rosea* Chloroform extract 75 mg/ml
Group 7: *R. rosea* Chloroform extract 100 mg/ml

Pheretima posthuma adult Indian earthworms (n=3, or 4) were placed in each Petri dish. The worms were observed for the time of loss of movement (paralysis), and death. Average time for the loss of movement (paralysis) and complete loss of movement even upon aggressive shaking (death) was recorded. Albendazole was used as the reference standard to compare the activity of the extract.

RESULTS:

GC–MS chromatogram of extract *R. rosea* clearly shows sixteen peaks representing the presence of 16 bio active compounds. Bioactive compounds from the extracts were identified from the data obtained in terms of the peak area, retention time and the molecular formula. (**Table 1**).

Table 1: GC–MS analysis of extract R.rosea

S. no	Peak name	Molecular formula	Molecular weight	Retention time	% Area
1	Lauroyl Peroxide	$C_{24}H_{46}O_4$	398	20.926	3.286
2	Sulfurous Acid, 2-Propyl Tridecyl Ester	$C_{16}H_{44}O_3S$	306	21.446	3.286
3	17-Pentatriacontene	$C_{35}H_{70}$	490	22.481	2.163
4	2-Octadecyl-Propane-1,3-Diol	$C_{21}H_{44}O_2$	328	23.097	3.070
5	Di-N-Octyl Phthalate	$C_{24}H_{38}O_4$	390	23.272	12.151
6	Di-N-Octyl Phthalate	$C_{24}H_{38}O_4$	390	23.432	9.051
7	Bicyclo[3.2.1]Oct-3-En-2-One, 3,8-Dihydroxy-1-Methoxy-7-(7-Methoxy-1,3-	$C_{21}H_{24}O_7$	388	23.552	8.440
8	1-Hexyl-1-Nitrocyclohexane	$C_{12}H_{23}O_2N$	213	23.812	2.075
9	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)-	$C_{30}H_{50}$	410	24.277	5.848
10	17-Pentatriacontene	$C_{35}H_{70}$	490	24.477	9.390
11	Sulfurous Acid, Octadecyl 2-Propyl Ester	$C_{21}H_{44}O_3S$	376	24.602	7.408
12	Hexacosyl Acetate	$C_{28}H_{52}O_2$	424	25.483	20.779
13	17-Pentatriacontene	$C_{35}H_{70}$	490	25.658	8.398
14	Ethyl Iso-Allocholate	$C_{26}H_{44}O_5$	435	26.613	2.162
15	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	$C_{20}H_{40}$	280	26.668	1.803
16	Hexadecane, 1-Chloro	$C_{16}H_{33}Cl$	260	26.808	2.259

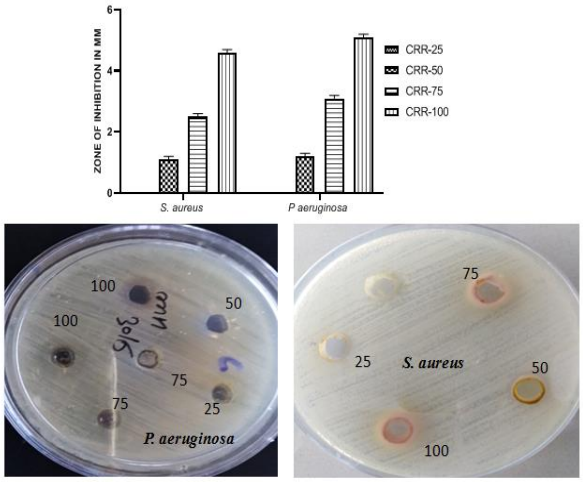

Figure 1. showing the anti-bacterial effect of *R. rosea* different concentration ns against *S. aureus* and *Pseudomonas aeruginosa*

The antimicrobial activity of extracts *R. rosea* were studied in different concentrations (25, 50,75and 100 µg/ml) against *S. aureus* and *Pseudomonas aeruginosa* pathogenic bacterial strains. The extract shows that varying degree of antimicrobial activity 50 and 100 µg/ml as shown in (figure1)

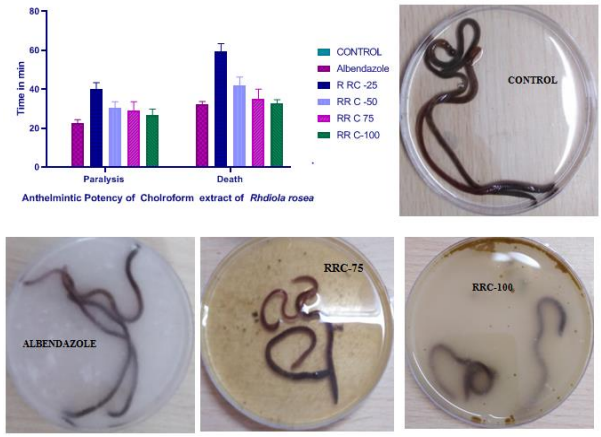

Figure 2. Showing anthelmintic activity *R. rosea*

The *R. rosea* extract was studied in different concentrations (25, 50, 75 and100 mg/ml) anthelmintic activity on *Pheretima posthuma* worms. Extract shows a significant activityin a dose dependent manner as shown in (Figures 2). The anthelmintic activity *R. rosea* extract in lower concentration not significant, in higher concentration extract 50,75 and 100 mg/ml exhibit significantly 35.41±3.85, 34.72± 3.15 and 32.15 ± 3.30 for paralysis time minute, in death time of the worms 42.98 ± 4.02 ,39.22 ± 4.12 minute and 38.06 ± 3.15 minute. Albendazole 22.75 ± 1.63 and 31.20 ± 1.25. Effectiveness of the extract *R. rosea* was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms.

DISCUSSION:

Resistance to antibiotics is of more significant concern worldwide due to rising incidences of resistance in the pathogens against the antimicrobials and antibiotics. This increase in the resistance is attributed to the random, indiscriminate use of the available antimicrobial drugs. Therefore, the scientists are continually looking for newer antimicrobial agents, particularly from the natural sources[10].

Natural sources, particularly the plants and herbs, have always been the scientist's first choice in the discovery and development of the therapeutic agents due to the presence of the phytoconstituent with various medicinal properties. Besides, medicinal agents obtained from plants are generally considered safe and are recommended for various ailments [11-12]. Many plants have been studied and are reported to contain the phytoconstituent effective against various pathogens[13].

Helminths are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease. Helminthic infections are commonly found in villages of developing countries and are being recognized as cause of much acute as well as chronic illnesses among humans [14-15]. Many helminthic infections occur in poverty-stricken and developing countries with warm, moist environments and poor sanitary conditions[16]. Although helminth infections can affect anyone, children in developing nations are mostly at risk. The World Health Organization reports a 35% infection rate for roundworm, which is a common parasitic worm[15]. These infections are usually transmitted through contaminated soil, food, or water; feces; and unwashed hands or contact with a contaminated object. There is a continuous search for alternative leads particularly from plants.

Phenylethanoids, phenylproponoids, and their glycosides, monoterpene alcohols and their glycosides, aryl glycosides, cyanogenic glycosides, proanthocyanidins, flavonlignans, flavonoids, and gallic acid derivatives, phenolic compounds tyrosol, salidroside, and rosavin derivatives are reported to be present in higher amount in the roots and rhizomes of *Rhodolia rosea* [4-5].

CONCLUSION

Rhodiola rosea has gained popularity as a medicinal herb and has been used in Chinese medicine for its adaptogenic properties. This research study was aimed at investigating the antibacterial and anthelmintic effect of *R. rosea* extracts. Besides, the extracts were evaluated for potential phytoconstituent effective against the microbes and helminth parasites. The extracts under study demonstrated a significant antibacterial and anthelminthic activity. Antibacterial and anthelminthic effects of the extracts may be ascribed to the presence of various phytoconstituent including alcohol derivatives, flavonoids, and phenolic compounds, a variety of glycosides, and the essential oils.

ACKNOWLEDGEMENT

Authors would like to acknowledge and thanks to Dr. Long chiau Ming, Associated Professor, Deputy Dean faculty of Pharmacy, Quest international university Perak for authentication of plant, we also thank and appreciate MEDICULE for scientific soloution Hyderabad, providing chemicals. We heartly gratitude to Prof Dr. Roshan. S Deccan school of pharmacy, Hyderabad for his valuable suggestion in research work.

FUNDING

No funding source for this project.

CONFLICT OF INTEREST

We have no conflict of interest to declare.

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