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STUDY OF THE ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF SOLVENT EXTRACTS OF *RHEUM WEBIANNUM* (REVANDCHINI)

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ABSTRACT

Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of some known drugs. Components with medicinal properties from plants play an important role in conventional Western medicine. Natural products and their derivatives represent more than 50% of the drugs in clinical use in the world. One of the paramount reasons for pursuing natural products chemistry resides in the actual or potential pharmacological activity to be found in alkaloids, terpenoids, coumarins, flavanoids, lignans, glycosides etc. Phytochemicals produced in plants are secondary compounds responsible metabolic activities and defense in purpose. Rheum webiannum is also known as Indian or Himalayan rhubarb and Revandchini. In the present investigation, the different solvent extracts viz. Ethyl acetate (ETA), Methanol (MET), Distilled water (AQ), Petroleum ether (PET) and Hexane (HEX) of rhizomes of *Rheum webiannum* were screened for antimicrobial and antioxidant potential 250 µg/ml. The potent antibacterial extracts viz. ethyl acetate, methanol and aqueous extracts showed least MIC values against the bacterial cultures. The results also showed the potent antioxidant potential of all the extracts. Amongst all the extracts, ethyl acetate and methanol extracts showed significant antioxidant potential. The study thus confirms the presence of antimicrobial and antioxidant compounds in the rhizomes of Revandchini.

KEYWORDS: *Rheum webiannum* (Rhubarb/Revandchini), solvent extracts, antimicrobial and antioxidant activity.

INTRODUCTION

Plants have been an integral part of the ancient culture of India as medicine, and their importance even dates back to the Neanderthal period. The use of plant-derived medicines in the treatment and prevention of disease has been documented over five millennia. Medicinal plants have been used throughout the world. However, their wide usage had been limited to China, India, Japan, Pakistan, Sri Lanka, Thailand and a number of African countries.[1] Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. These antimicrobial compounds isolated from plants are found to be very effective in comparison to traditional medicines and produces no side effects. The biological activity of many plants has been known through scientific research and any literature search via the internet or elsewhere, would reveal that numerous new publications are added to the scientific literature every day. Many efforts have been made to discover new antimicrobial or drug from various sources such as microorganism, animals and plants. Systematic screening of them may result new findings, drug. Considering the country's vastness with its varied flora and the utilization of the plants as traditional medicines, these studies are rather meager. Himalaya represents one of the most important mega centers of biodiversity, showing 50% of the vegetational wealth of the Indian subcontinent. Several plants exert antimicrobial activity; however, this effect is much more pronounced when its crude extracts or purified components are studied. Most available data about the antimicrobial effect of a herb or plant extract are based on *in vitro* studies, but several herbs are used due to its antimicrobial effects in the complementary or ethno-medicine based on empirical application *in vivo.*[2] Previous study[3] revealed that the ethno medical use of herbal remedies for *Mycobacterium* related infection strongly correlated with their *In vitro* antibacterial activity against *Mycobacterium tuberculosis.* Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and selected on the basis of their ethno–medicinal use.[4] The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. Medicinal plants have always provided a stable source for medicines not only the herbs themselves but certain plant-derived compounds have served as lead molecules for further chemical modulation and natural products still continue to play a highly significant role in drug discovery and development process.[5] The present investigation deals with the determination of antimicrobial activity and antioxidant activity of polar and non polar solvent extracts of rhizomes of *Rheum webiannum* (Revandchini) at 250 µg/ml.

EXPERIMENTAL

Plant material

The plant parts (rhizomes) of *Rheum webiannum* were collected from local gardens. The voucher specimens of plant material were stored in the institute herbarium for future reference. The collection took place in the flowering season of year 2016. The rhizomes were dried in the shade in an open air for 5-10 days and ground to form fine powder.

Preparation of plant extracts

The method[6] was adopted for preparation of plant extracts with little modifications. Briefly 20 g portions of the powdered plant material was soaked separately in different solvents i.e. hexane (HEX), petroleum ether (PET), methanol (MET), ethyl acetate (ETA) and distilled water (AQ) on the basis of increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30 ^{0}C.

Determination of antimicrobial activity

Culture Media

For antibacterial activity, Soyabean casein digest agar/broth and antifungal activity, Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India were used.

Inoculum

The bacteria were inoculated into Soyabean casein digest broth and incubated at 37 ^{0}C for 18 h and suspension was checked to provide approximately, 10^5 CFU/ml. The same procedure was done for fungal strains and there strains were inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48-72 h.

Microorganisms used

The pure cultures of test microbes, *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger and Candida albicans* were used for the study.

Determination of diameter of zone of inhibition by well diffusion method

The agar well diffusion method[7] was modified. Soyabean casein digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the

bacteria separately suspended in nutrient broth. Sabouraud's dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and blanks. Sterilized N-saline solution was used as the negative control. Standard antibiotic (Erythromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were then incubated at 37 ^{0}C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. For assaying, antifungal activity of plant extracts, Sabouraud's dextrose agar/ broth medium plates were used. The same procedure as that for determination of antibacterial property was adopted and then after the diameter of zone of inhibition was observed after 48-72 h. Fucanazole (1 mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

Determination of Minimum Inhibitory Concentration (MIC)

MIC value of potent plant extracts against the concerned pathogen was determined by the method adopted[8,9] with some modifications. Plant extract (s) were prepared in highest concentration (250 µg/ml) in sterile distilled water and is serially diluted with N-saline (0.85% NaCl) and 100 µl of bacterial suspension was added to different test tubes and incubated for 48 h. The inhibition of turbidity appeared in the minimum dose at which total growth of bacteria gets killed is known as minimum lethal concentration (MLC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of bacteria is known as minimum inhibitory concentration (MIC).

Determination of antioxidant activity

Estimation of Total phenolic content (TPC) of extracts

The total phenolic content of each extract of the plant material was determined by the method.[10] The phenolic content was expressed as mg/g gallic acid equivalents. In brief 100 µl aliquots of the sample were added to 2 ml of 0.2% (w/v) Na_2CO_3 solution. After 2 minutes of the incubation. 100 µl of 500 ml/1 Follin-Ciocalteu reagent was added and the mixture was allowed to stand for 30 minutes at 25^{0}C. The absorbance was measured at 750 nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The total phenolic content (TPC) was determined using the standard gallic acid calibration curve.

Determination of antioxidant activity by DPPH radical scavenging method

The extract solution for the DPPH test[11] was prepared by re-dissolving 0.2 g of each of the dried crude extract in 10 ml of the specific solvent in which the extract was prepared. Two ml of the DPPH solution was mixed with 20 µl of the plant extract solution and transferred to a cuvette. The reaction solution was monitored at 515 nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible Systronics spectrophotometer.

The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation.

Inhibition%= (Abst=0 minutes- Abst=30 min)/ Abst=0 minutes ×100.

Where, Abst=0 minutes was the absorbance of DPPH at zero time and Abst=30 min was the absorbance of DPPH after 30 minutes of incubation. Ascorbic acid (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of plant extract solution to the ascorbic acid equivalent. IC_{50} is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Determination of Superoxide anion radical Scavenging Activity

Superoxide anion radical scavenging activity was measured with some modifications[12] The various extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 uM NBT separately. The reaction solution was illuminated by exposure to 30 W fluorescent lamps for 20 minutes and the absorbance was measured at 360 nm using a spectrophotometer. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control. The superoxide anion radical scavenging activity (%) was calculated as.

$$\frac{A_0 - A_S}{A_0} \times 100$$

where, A_0 = absorbance of positive control; A_S = absorbance of sample

Determination of Total antioxidant Activity

Total antioxidant activities of extracts and ascorbic acid were determined. An aliquot (0.1M) of the extracts were combined with 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95 ^{o}C for 90 minutes. After that the sample were cooled at 25^{o}C, the absorbance was measured at 695 nm against blank. The blank contained 1ml of reagent solution without

sample. The total antioxidant activity was expressed as an absorbance value at 695 nm. Higher absorbance value indicates the maximum antioxidant activity.

RESULTS

The powdered rhizomes extracts of *Rheum webiannum* were prepared in different solvents used according to increasing polarity at a concentration of 250 µg/ml. The antimicrobial activity was determined by well diffusion method. Ethyl acetate, methanol and aqueous extracts of the rhizomes of the plant showed significant antimicrobial activity against the pathogens used for the study viz. *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger and Candida albicans.* It was found that, amongst all the pathogenic cultures, *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa and Staphylococcus aureus* were the sensitive pathogens against both polar and non polar extracts while *Aspergillus niger* and *Candida albicans* were found to be the most resistant pathogens studied against both polar and non polar solvent extracts. The level of significance was found to be $p<0.05$. The results are shown in Table 1 and Figure 1 (a) & (b). The potent antibacterial extracts viz. ethyl acetate, methanol and aqueous extracts showed least MIC values against the bacterial cultures. The results of MIC are shown in Table 2 and Figure 2. It was found that, ethyl acetate is the significant and most appropriate solvent for extraction of antioxidant molecules as the antioxidant activity determined by different assays was found to be maximum in ethyl acetate extract in comparison to methanol and aqueous extracts. Also it was suggested that, hexane extracts are not significant to extract antioxidant molecules in comparison to petroleum ether extract as lowest antioxidant activity was found in the hexane extract. The antioxidant activity of the extracts thus follows the order ethyl acetate>methanol>aqueous>petroleum ether>hexane.

The results showed that, TPC in ethyl acetate, methanol and aqueous extract were found to be 218, 176 and 153 µg/g gallic acid equivalents followed by petroleum ether and hexane extracts viz. 126 and 95 µg/ml (Table 3; Figure 3). IC_{50} values of ethyl acetate, methanol and aqueous extracts were found to be 25.32, 31.18 and 37.45 µg/ml followed by 45.43 and 56.67 µg/ml of petroleum ether and hexane extracts respectively as determined by DPPH free radical scavenging activity. The results suggested that lower the IC50 values, dominant is the antioxidant activity (Table 4; Figure 4). With reference to superoxide anion radical scavenging method, polar extracts showed 75.56- 82.41% inhibition of superoxide followed by non polar extracts having 65.12- 74.32% inhibition (Table 5; Figure 5). Total antioxidant

activity also followed the same order (Table 6; Figure 6). Ascorbic acid was used as the standard antioxidant having IC_{50} value 55.45µg/ml in DPPH radical scavenging method and 87.80% inhibition of superoxide.

Table 1: Antimicrobial activity of solvent extracts of rhizomes of *Rheum webiannum*.

S.No.	Solvent extracts (250 µg/ml) /Positive controls (1mg/ml)	Diameter of zone of inhibition (mm)					
		Bacillus subtilis	***Micrococcus luteus***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Aspergillus niger***	***Candida albicans***
1.	**Hexane (HEX)**	10±0.56	10±0.54	5±0.87	7±0.95	NA	NA
2.	**Petroleum ether (PET)**	12±0.34	11±0.45	8±0.63	10±0.53	NA	NA
3.	**Methanol (MET)**	22±0.04*	24±0.045*	27±0.03*	25±0.04*	NA	NA
4.	**Ethyl acetate (ETA)**	25±0.025*	28±0.02*	32±0.025*	26±0.045*	NA	NA
5.	**Distilled water (AQ)**	23±0.035*	15±0.03*	21±0.024*	16±0.04*	NA	NA
#	**Erythromycin**	32±0.02*	26±0.025*	25±0.03*	28±0.02*	NT	NT
#	**Fucanazole**	NT	NT	NT	NT	25±0.04*	28±0.025*

*Level of significance, $p<0.05$; NT, Not tested; NA, No activity

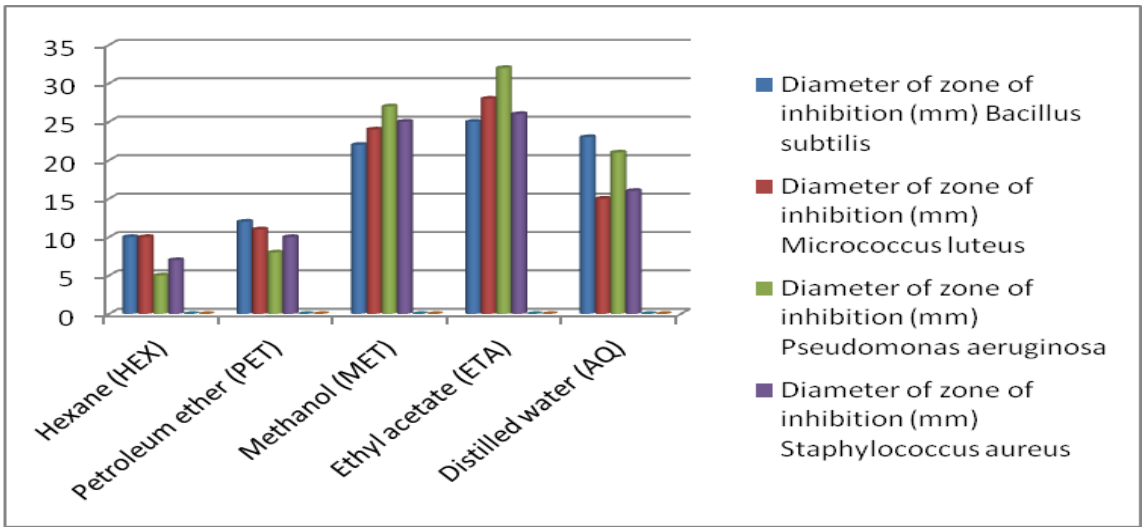

Figure 1 (a): Graphical representation of antimicrobial activity of solvent extracts of rhizomes of *Rheum webiannum*.

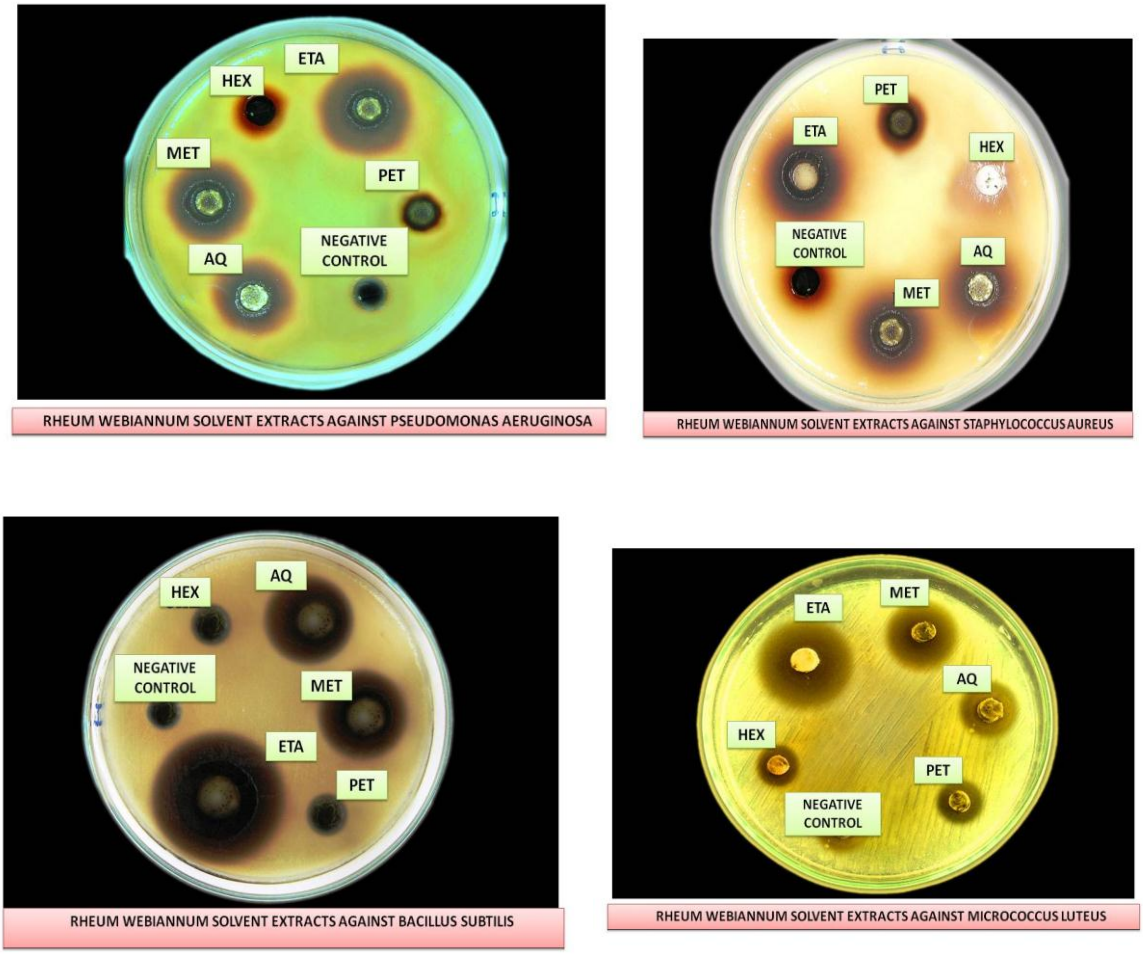

Figure 1 (b): Antimicrobial activity of solvent extracts of rhizome of *Rheum webiannum*.

Table 2: Minimum inhibitory concentration (MIC) of the potent plant extracts against specific bacterial cultures.

S. No.	Solvent extracts	MIC (µg/ml) against bacterial cultures			
		Bacillus subtilis	***Micrococcus luteus***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
1.	Hexane (HEX)	220	220	230	245
2.	Petroleum ether (PET)	225	230	240	230
3.	Methanol (MET)	150	130	120	140
4.	Ethyl acetate (ETA)	180	160	120	150
5.	Distilled water (AQ)	180	230	80	90

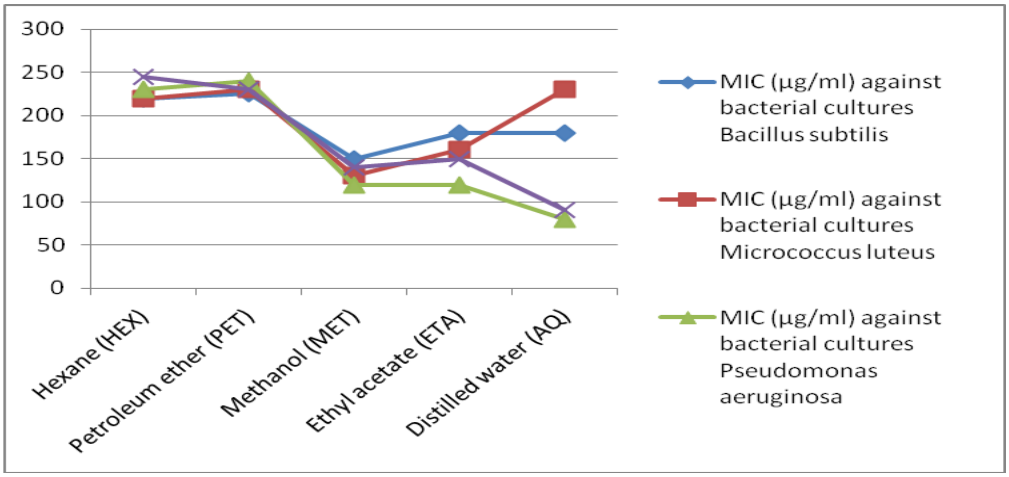

Figure 2: MIC of the potent solvent extracts against the respective bacterium.

Table 3: TPC (µg/g gallic acid equivalents) of solvent extracts of *Rheum webiannum.*

Rhizomes extracts	TPC (µg/g gallic acid equivalent)
Ethyl acetate extract (EA)	218
Methanol extract (MET)	176
Aqueous extract (AQ)	153
Petroleum ether extract (PET)	126
Hexane extract (HEX)	95

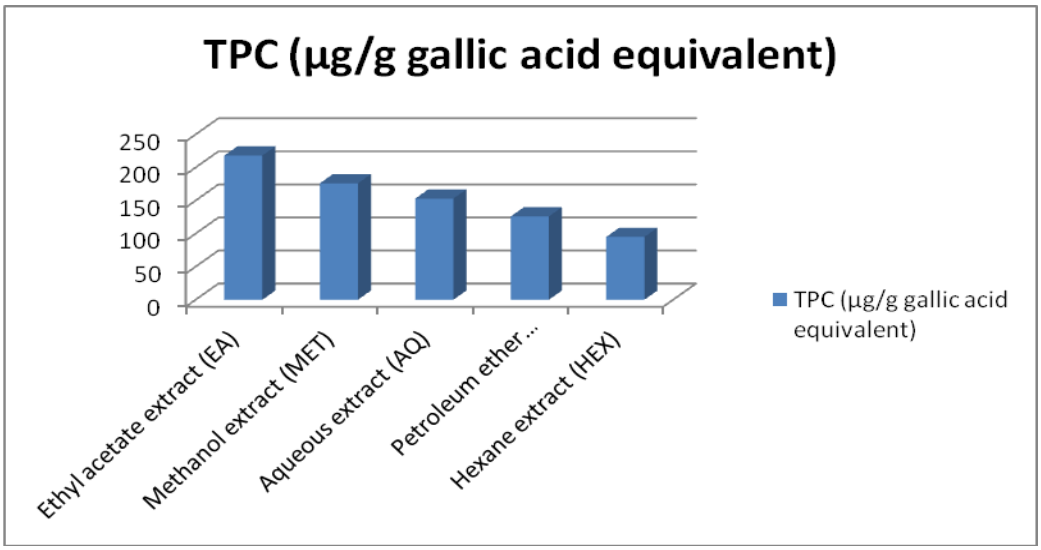

Figure 3: Graphical representation of TPC of solvent extracts of *Rheum webiannum.*

Table 4: IC50 values of solvent extracts of *Rheum webiannum.*

Rhizomes extracts	IC50 (µg/ml)
Ethyl acetate extract (EA)	18.56
Aqueous extract (AQ)	21.34
Methanol extract (MET)	26.78
Petroleum ether extract (PET)	32.24
Hexane extract (HEX)	56.45

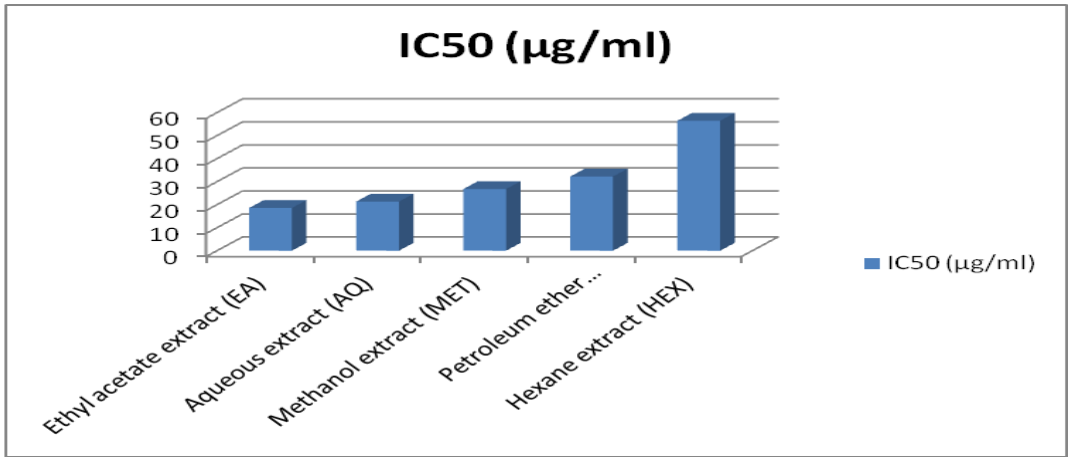

Figure 4: Graphical representation of IC50 values of solvent extracts of *Rheum webiannum*.

Table 5: Percent inhibition of superoxide anion radical scavenging activity of solvent extracts of *Rheum webiannum*.

Rhizomes extracts	Percent inhibition of superoxide anion radical scavenging activity
Ethyl acetate extract (EA)	75.56
Aqueous extract (AQ)	64.45
Methanol extract (MET)	72.0
Petroleum ether extract (PET)	52.21
Hexane extract (HEX)	24.56

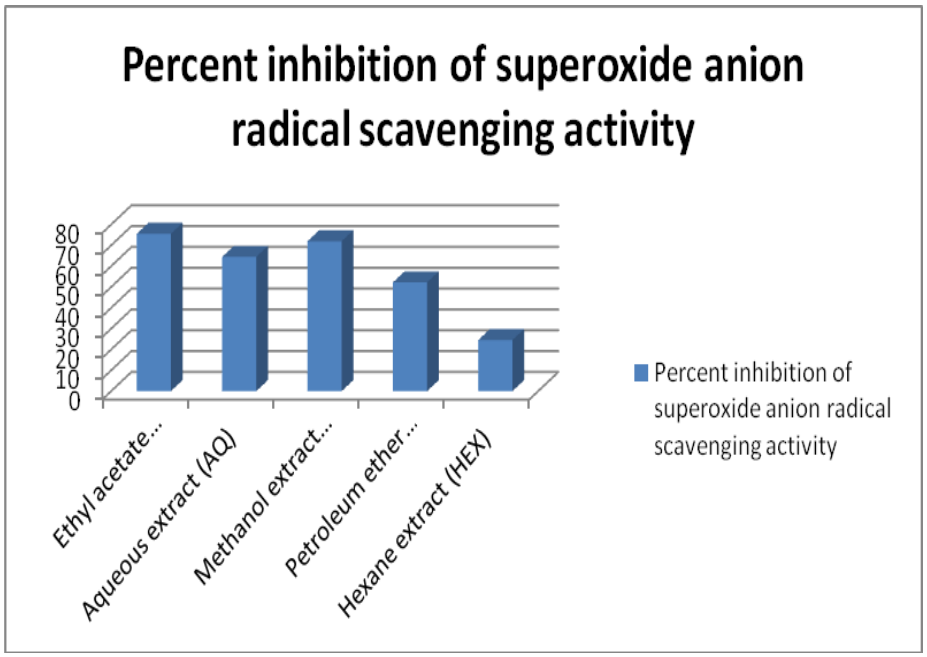

Figure 5: Graphical representation of percent inhibition of superoxide anion radical scavenging activity of solvent extracts of *Rheum webiannum*.

Table 6: Total antioxidant activity of solvent extracts of *Rheum webiannum*.

Rhizomes extracts	Total antioxidant activity (Absorbance values @ 695 nm)
Ethyl acetate extract (EA)	0.095
Aqueous extract (AQ)	0.075
Methanol extract (MET)	0.078
Petroleum ether extract (PET)	0.056
Hexane extract (HEX)	0.032

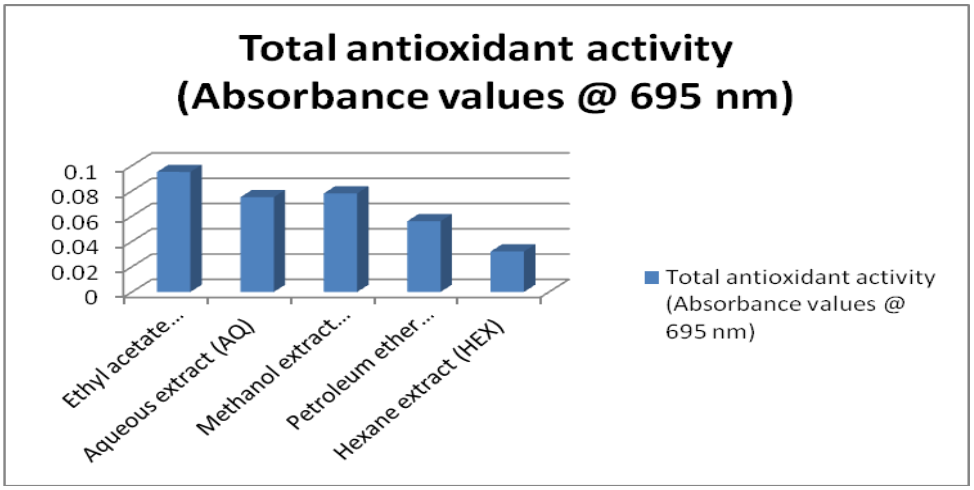

Figure 6: Graphical representation of total antioxidant activity of solvent extracts of *Rheum webiannum*.

DISCUSSION AND CONCLUSION

The present study suggests the antimicrobial and antioxidant potential of rhizomes of *Rheum webiannum* (Revandchini). The study revealed the potent effect of polar extracts in comparison to non polar extracts against the bacterial cultures. The results of the study correlate the previous findings reported by our group on another species of Rheum.[13-15] The study thus illustrates antimicrobial importance of rhizomes of the plant. Further studies can be carried out in order to isolate the active principles from the potent extracts of rhizomes which can be utilized to formulate an antimicrobial drug or can be utilized as the active ingredient in the formulation for designing the new anti-microbial drug.

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