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ESTIMATION OF BIOACTIVE COMPOUND USING RP-HPLC AND ANTIBACTERIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *SWERTIA CHIRAYITA* (ROXB. EX FLEMING)

Ankita Shrivastava[1], Rashmi Mishra[1], Malika Pal[1], Prabhat Jain[2]

[1]SAM Girls College, Bhopal (M.P.)

[2]Scan Research Laboratories, Indrapuri, Bhopal (M.P.)

*Corresponding Author's E mail: shrivastava.jannat@gmail.com

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ABSTRACT

Utilization of herbs for medicinal purpose started in the early history of mankind several thousand years ago. The worldwide increase of multidrug resistance has impaired the current antimicrobial therapy and warranting the search for other alternatives. *Swertia chirayita* is one of the potential medicinal plants of the family Gentianaceae in traditional medicine. Due to its high demand and scarcity, trade of chirayita is affected by adulterants. The present study was undertaken to find the antibacterial activity, phytochemical presence and quercetin was detected in hydroalcoholic leave extract of *Swertia Chirayita* under study by using RP-HPLC analysis. Hydroalcoholic leave extract of *Swertia Chirayita* were evaluated for antibacterial activity against Two-gram negative strain like Salmonella *bongori*, *Proteus Vulgaris* and one-gram positive strain like *E. Faecalis* using agar diffusion method (well method). The significant antibacterial activity of the active extract was compared with standard antibiotic ciprofloxacin (10-30 µg/ml). The antibacterial activity was determined by measuring the diameter of the zone of inhibition in term of millimeter (mm). The preliminary phytochemical studies and quantitative analysis of flavonids were performed by well reported method. The phytochemical analysis showed the presence of tannins, glycosides, flavonoids and alkaloids ect. It is concluded that the antibacterial activity showed by the plant is due to the presence of these phytochemicals. For future studies, phytochemcials responsible for these activities can be isolated and modified for pharmacological purpose.

K**eywords:** *Swertia Chirayita*, Quercetin, RP-HPLC, Antibacterial Activity, Flavonoids.

INTRODUCTION:

Antibiotics are one of the most important armaments for fighting bacterial infections and have played a fundamental role in improving the quality of human life since their introduction [1]. However, in current years, many commonly used antibiotics are proving to be less effective due to appearance of antibiotic resistance [2]. Antibiotic resistance is the ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once sensitive. The spread of antibiotic resistance as well as the evolution of new strains of disease causing agents is of great concern to worldwide health [3]. Hence, it is very important, to discover new drugs for infectious diseases with lesser or no bacterial resistance at all. Since, plants are rich in a wide variety of secondary metabolites, therefore, the use of herbal medicines as alternative therapy for infectious diseases has been

intensified due to their high content of antimicrobial agents such as polyphenols, i.e. tannins, alkaloids, flavonoids and terpenoids [4, 5]. Most medicinal preparations or drug were derived from natural sources or plant, whether in the simple form of raw plant materials or the refined form of crude extracts, mixtures, etc. In present world, medicinal plants are gaining attention owing to the fact that the herbal drugs are cost-effective, easily available and with little or no side effects and play a major role in the prevention and treatment of various human diseases. Plant-based natural constituents can be obtained from any part of the plant like bark, leaves, flowers, fruits, roots, seeds, etc. Recently, the quest for the isolation of new compounds from medicinal plants has become an interesting area of research [6]. Herbs are widely exploited in traditional medicine and their curative potentials are well documented. About 65% of new drugs developed between 1980 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious diseases [7]. In traditional system of medicine, plants have been used since ancient times for prevention from infectious diseases. Thus, the modern system of medicine is becoming increasingly interested to the use of antimicrobial and other drugs derived from plants, as most of the existing antibiotics have become ineffective due to enhanced drug resistance [8]. Another dynamic factor for the changed interest in plant antimicrobials in the past 25 years has been the rapid rate of plant species extinction [9]. Hence, it is all the more necessary that the impressive array of knowledge assembled by indigenous people about plants, should be explored scientifically to be of continued use in our worldwide health campaign [10]. The plant *Swertia chirata* Ham. (F. Gentianaceae) is a tropical family of small trees, herb and bitter tonic. It consists of 180 species. About 8-10 species exist in India [11]. This plant is indigenous to temperature Himalayas at altitudes above 4000 feet from Kashmir, Bhutan and Nepal [12]. The main chemical constituents of this plant are two bitter principles, *viz*, ophelic acid, an amorphous bitter principle and chiratin, a yellow bitter glucoside. The plant also contains resins, tannin, gum, carbonates, phosphates and 4-6% ash, lime and magnesia [13, 14]. A number of workers have shown that the plant contains bitter glucosidal components, chiratin and amarogentin, swerchirin, gentiopicrin, phytosterd and also a number of acid, yellow crystalline phenols and saccharine [15-17]. It has anti-microbial activity against Gram positive and Gram negative bacteria. All the plant are used as astringent, unani-tonic to heart, liver, eyes, cough, scanty-urine, melancholia, dropsy, sciatia, skin diseases, the plant is used as a bitter tonic in gastrointestinal disorders, like dyspepsia/anorexia, it is used as digestive, febrifuge and laxative. It is used to prevent malaria, particularly useful in fever. The plant is also effective against intestinal worms burning of the body, bronchial asthma, regulating the bowels [18]. The study was designed to demonstrate the potent antibacterial activity against gram positive and gram negative bacteria. Total flavonoid content was determined spectrophotometrically as well as characterised by using HPLC.

MATERIALS AND METHODS

Plant material

The leaves of *Swertia Chirayita* were collected from ruler area of Bhopal (M.P.) in the month of Feb, 2017. The leaves plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf was air dried under room temperature.

The dried plant samples were cut and grinded to make it in powder form.The powdered samples were stored in clean, dry and sterile container for further use.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade from Merck Ltd, New Delhi, India.

Extraction procedure

The shade dried material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The extraction was done using Soxhlet extractor which is a versatile tool that can be used to separate a single gram to hundreds of grams with near 100% recovery. 50 gm of dried defatted powdered sample are packed into thimble of the Soxhlet Apparatus. 200 ml of hydroalcoholic solvent was added to the thimble. Then the apparatus was operated and continued for 48 hours regularly monitoring the circulation of water in the condenser. After warm extraction the solvent was removed using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts. The non-soluble portion of the extracted solid remained in the thimble was discarded [19-20].

Qualitative phytochemical analysis of plant extract

The *Swertia chirayita* leave extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate, Khandelwal and Ayoola *et al* [21-23]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid.

Total flavonoids determination

The total flavonoid content was determined using the method of Atanassova *et al* [24]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extracts or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Quantification of flavonoid compounds by HPLC technique

For HPLC investigation of flavonoid compounds the hydroalcoholic extracts of *Swertia chirayita* leave under study were used as a preliminary assessment of various compounds. The HPLC apparatus used for analysis was composed of a waters equipped with a UV dual detector and generated data were analyzed

using Waters Ace software. For chromatographic separation Thermo C18 column (250 X 4.6 mm, 5µm) was applied. The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL/ min. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm. Sample volume (20 uL) and analysis time was 15min for both, standards and samples used for analysis. A quercetin was used as standards. A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}.

Antibacterial activity of *Swertia chirayita* extract

The well diffusion method was used to determine the antibacterial activity of the extract prepared from the *Swertia chirayita* using standard procedure of Bauer *et al* [25]. The drug used in standard preparation was ciprofloxacin of IP grade. The antibacterial activity was performed by using 24hr culture of *S. Bongori, E. Faecalis and P. Vulgaris.* There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The diameter of zone of inhibition of each wall was recorded.

RESULTS AND DISCUSSION

The crude extracts so obtained after the hot continuous percolation extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant. The yield of extracts obtained is depicted in the Table 1.

Table 1: Percentage Yield of Extract of *Swertia chirayita*

S. No.	Solvents	Percentage Yield (%)
1.	Hydroalcoholic	5.3

Phytochemical analysis of hydroalcoholic extracts of leaf sample of *Swertia chirayita* showed the presence of flavonoid, amino acid, protein, carbohydrate and diterpines while, alkaloid, glycosides, phenols and saponins were not detected. This study indicates the presence of flavonoids present in

sufficiently enough quantity in extract so flavonoid is the phytochemicals that are present in hydroalcoholic extract Table 2.

Table 2: Phytochemical Screening of *Swertia chirayita* Extract

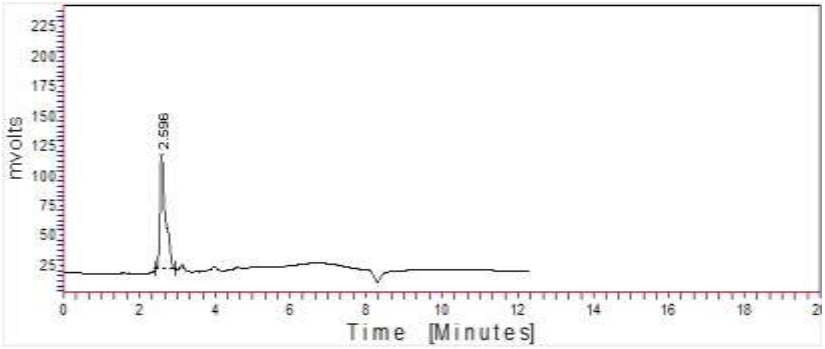
S. No.	Constituents	Extract
1.	Alkaloids	-
2.	Glycosides	-
3.	Flavonoids	+
4.	Diterpenes	+
5.	Phenolics	-
6.	Amino Acids	+
7.	Carbohydrate	+
8.	Proteins	+
9.	Saponins	-
10.	Oils and fats	-

The total flavonoid content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extracts of leaves of *Swertia chirayita* showed the content values of 0.903 mg/100mg (QE).

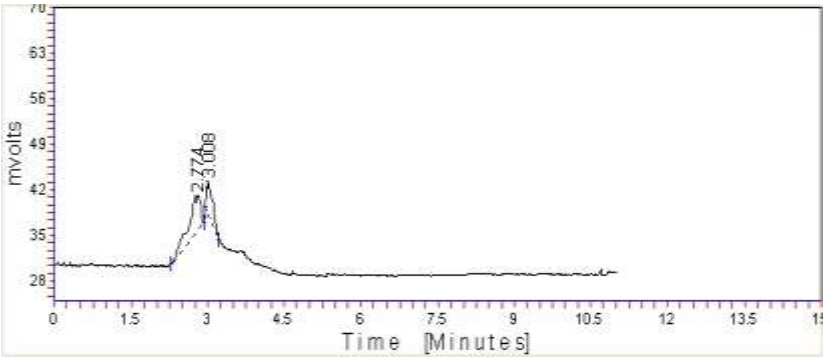
Flavonoids compounds are secondary metabolites in plants which play an immensely important role in human health and nutrition. The HPLC chromatogram of standard quercetin and hydroalcoholic extract are shown in Figure 2 and the values are expressed in ppm. The retention time for standard and extract was found to be 2.596 min and 2.774 min respectively. Characteristics parameters for standard quercetin and results of quantitative estimation of quercetin in hydroalcoholic leaf extract were given in table 4.

Table 4: Quantitative estimation of Quercetin in extract

S. No.	Extract	RT	Area	% Assay
1.	***Swertia chirayita***	2.774	82.515	0.1196



(A)



(B)

Figure 2: Chromatogram of (A) Standard Quercetin (B) Hydroalcoholic Extract of *Swertia chirayita*

The antibacterial activity of hydroalcoholic leave extract of *Swertia chirayita* showed bioactivity by inhibiting growth of microbial species selected for the test as shown in Figure 3 and 4. The zone of inhibition shown by the extracts was comparable to the standard antibiotics. It is effective against *Salmonella bongori*, *E. Faecalis* and *Proteus Vulgaris* in concentration dependent manner.

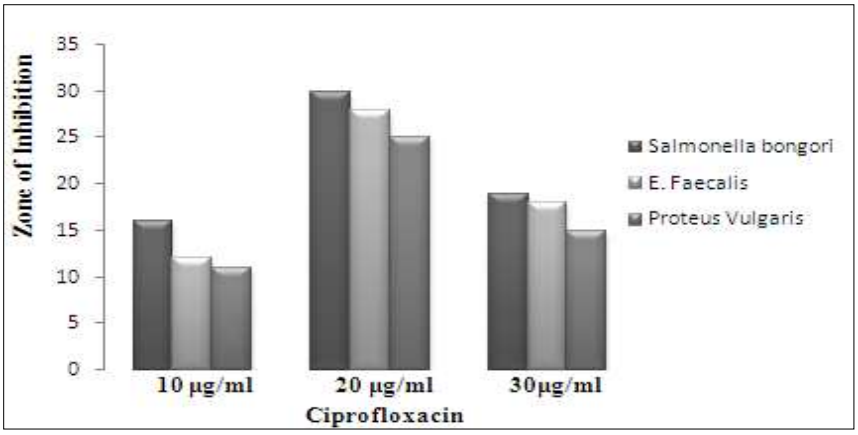


Figure 3: Zone of inhibition of standard ciprofloxacin against different bacteria

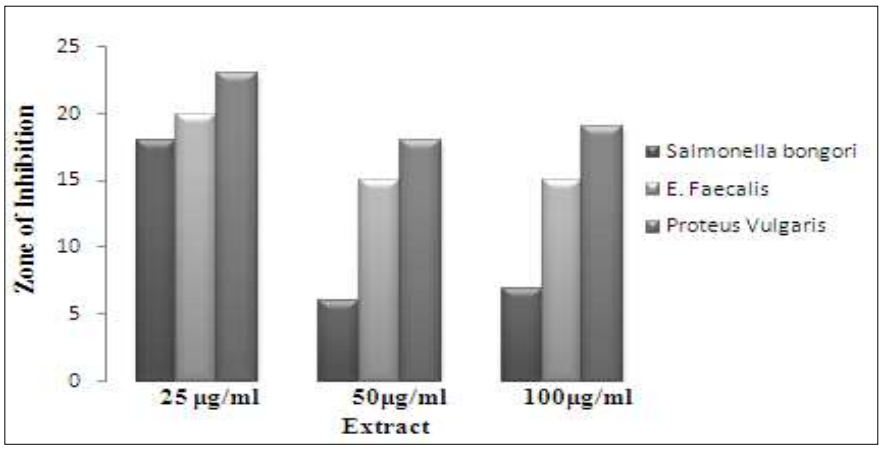


Figure 4: Zone of inhibition of hydroalcoholic extract of *swertia chirayita* against different bacteria

CONCLUSION

Hydroalcoholic leave extract of *Swertia chirayita* was possess antibacterial potential against both gram positive and gram negative bacteria due to the presence of various phytochemical constituent. This natural product can bring new and effective antimicrobial agents and serve as alternate source of combating infections in human beings. Hence this research can have a promising potential in various traditional, complementary and alternate systems of treatment of human diseases. Although antibacterial activities of the mentioned extracts were lower than standard reference compounds, this needs to be fully clarified by further assay methods and using additional concentrations of extracts. Further phytochemical studies using various biochemical and molecular biology tools are also required to isolate and characterize active ingredients that are responsible for its antibacterial activity and to explore the existence of synergism if any, among the compounds.

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