*Official Publication of Pakistan Society of Biomass and Bioenergy*
**Journal of Biomaterials and Bio Products Technology**
ISSN: 2790-2595 (Print), 2790-2609 (Online)
http://www.jbbt.org

# CHERMICAL ANALYSIS OF SWERTIA CHIRAYITA AND THE IN VIVO ASSESSMENT OF ITS BIOACTIVITY

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## ABSTRACT

Swertia chirayita is medicinal plant which is widely used by Ayurvedic physicians because it contain many natural ingredients which are useful to cure many diseases.A study conducted to quantify the phytochemical from the plant. The Antioxidant activities of various extracts assessed by using DPPH assay while antimicrobial activities evaluated against bacterial strains by comparing with standard antibiotics.Finally on the basis of phytochemicals available in plants its bioactivity correlated further for its scientific use and development of lead compounds those required for human ailments.

**Keywords:** Phytochemicals Antioxidant, Antimicrobial, Medicinal plants

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## INTRODUCTION

The basic needs of primary health care system depends on availability of different drugs in the market. Traditional medicine is still the most manageable and affordable source of treatment. Different diseases treated by the medicinal plants in better way, either in the form of pure active principles or natural ways and sporadically used curative

tonic in the developing countries. In the family Gentianaceae Swertia, a genus which comprise a huge group of annual and perennial herbs, expressing about 135 species. Swertia species have common ingredients for number of herbal remedies ( Abbasi et al., 2915; Adedapo et al., 2009 ).Recorded 40 species of swertia in india, out of which chirayita is the most beneficial for its therapeutic actions. In 1993 Edward showed that prevalent uses of chirayita in traditional medicine give rise to over-exploitation from the natural environment. Ethnomedicinal studies identified swertia most bitter herb in its taste due to the existence of different chemical ingredients ( Newman and Crag, 2012).

The swertia chirayita used as a drug constitutes whole plant in dried form collected in its flowering stage. The chirayita is known as a trade name and also the local name of the plant.

Swertia is well known for swiftly lowering fevers from colds and flues particularly malarial fever ( Adedapo et al., 2009). Swertia is a powerful anti-inflammatory agent making it good for joint disease, swelling, pain, and redness. Swertia is excellent remedy for rheumatoid arthritis.

Swertia also works well for treating liver cihrosis and also does a good job of detoxing the liver. Swertia also increases metabolism thus helping with weight loss(Beauchamp and Fridovich,1971)

. Swertia has good antidiabetic ,antianemic properties.Swertia species have xanthones as secondary metabolites which are used effectively to treat malarial fever and tuberculosis (Cefafelli et al., 2006; Demirtas et al., 2009). Scientists study antimicrobial activity of plant extracts when they agreed that conventional medicine can act as a substitute of healthiness as well as used as antibiotic (Dillard and German,2000) . Therefore keeping in view the importance of this herb current study designed to evaluate

the phytochemical evaluation of swertia chirayita and invivo assessment of its bioactivity (Dufour et al.,2000; Flohe and Gunzler,1984).

## MATERIALS AND METHODS

### Collection and preparation of plant samples

Samples of swertia chirayita were collected from hilly area of Murree, in fine plastic bags duly labelled with data and time of collection of samples . After transportation of samples to laboratory , samples were properly identified by expert taxonomist and specimen was deposited for future reference (voucher no. 137 ). Samples were subjected to shad dried follow by sun and oven drying at lower temperature. Dried samples were ground to powder form and stored at lower temperature till further uses (Newman and Cragg,2012).

### QUALITATIVE ANALYSIS OF PHYTONUTRIENTS:

Qualitative assessment of flavonoids, alkaloids, phenols, tannins, saponins glycosides, terpenoids and steroids was carried out in the precise ways ( Harborne, 1998).

### Test for alkaloids

Sample of 0.5 to 0.6 g finally mixed with 1%HCL of 8ml, filtered. Filtrate of 2 ml were taken and reacted with reagents ( Abbassi et al., 2015 ), turbidity or precipitation showed the presence or absence of alkaloids.

### Test for proteins.

**a) Ninhydrin test**: Crude extracts for protein test was heated with 2 ml of 0.2 % Ninhydrin solution and violet coloration specified the occurrence of proteins and amino acids.

### Test for reducing sugars

**a) Fehling's test**: Fehling A and Fehling B reagents combine by equal parts and 2 ml from infusion taken further added to extract and heated till boiling. Reducing sugars identified by the appearance of reddish precipitate.

**Test for carbohydrates**

**a) Benedict's test**: Benedict's reagent of 2ml added in the sample and heated till boiling, magenta brownish precipitate seen showed the occurrence of the carbohydrates.

**b) Molisch's test**: Sample was made with 2.5 ml of Molisch's reagent and , 2 ml of concentrated $H_2SO_4$ was transferred to the test tube. violet ring will be formed at the junction of test tube indicating carbohydrate presence.

**Test for Iodine**

Iodine solution of 2ml added in the prepared sample. A dusky dark blue pigmentation showed the iodine indication.

**Test for Saponin**

Sample of 0.4-0.5 g was mixed in hot water in the test tube. Test tube will show mixture vigorously forming foam and the elevation of the foaming was noted to define the saponin amount in our sample. 3 drops of olive oil was added to the frothing and mixed strongly for the development of suspension , representative of saponins.

**Test for flavonoids**

**a. Alkaline reagent test**:

A solution of 2% of NaOH was added to Crude extracts of samples. Only few drops of acid give rise to yellowish colour mixture change into colorless form, directed the characteristic of flavonoids ( Harborne, 1998).

**ANTIOXIDANT ASSAY**

**DPPH radical scavenging activity assay**

Each sample of 4mg prepared and its stock prepared in DMSO in different concentrations. 2.4mg of DPPH taken and made its volume upto 100 ml in methanol .

Extract was tested first at single concentration and those showing good antioxidants activity were tested for different range of concentration to establish the EC50.100 $\mu$l of each dilution was taken in test tube along with 2ml of DPPH and one test tube was taken as blank .Negative control contain all reagent except extract. After 30 min absorbance measured at 517nm,experiment done in triplicate and ascorbic acid used as standard (Skehan et al., 1990 : Valko et al., 2006; Wu et al., 2006).

The ability of sample to scavenge DPPH radical calculated by following formula: DPPH Scavenging activity % = (A. control - B. sample) / (A. control)*100

**ANTIMICROBIAL ACTIVITY:**

**Preparation of extract for antimicrobial activity**

Preparation of samples extract for antimicrobial activity was defatted with petroleum ether and then consecutively prepared with water and methanol solvents with the help of soxhlet tool. The extracts we prepared then cooled at 25C,Puted for evaporation until sample completely dried and finaly got filtrate. The extracts we used for analysis dissolved in dimethylsulfoxide (DMSO) for antimicrobial assay with minor alterations ( Upadhyay, 2015).

**Test Organism**

**A** panel of test organisms was used including Echerichia coli and gram positive Staphylococcus aureus,Pseudomonas aeruginosa, and streptococcus pyogenes were procured from National Institute of Health Islamabad.

**Culture Media:**

The antibacterial and antifungal studies were carried out using nutrient agar medium followed by refrigeration storage at $4^0$C.bacterial strain grown at $37^0$C maintained at nutrient agar slant at $4^0$C.

**EVALUATION OF ANTIBACTERIAL**

**ACTIVITY:**

The well diffusion assay technique ( Upadhyay, 2015) used to evaluate the in vitro antimicrobial activity of all samples poured at 10$\mu$l of microbes culture added to petri plates.After media solidified hole was made by using 5mm cork and hole filled with20$\mu$l of plant extract(10mg/ml and 20mg/ml).The inoculated plate left for refrigeration and then put in incubator for incubation at $37^0$C for 24 hrs .

**RESULTS AND DISCUSSION:**

Qualitative analysis of swertia chirayita showed the presence of alkaloids carbohydrates,flavonoids,glycosides,tanins/phenols,saponins and proteins but tanins found absent in chirayita sample 2 by ferric chloride and lead acetate test (Dillard and German, 2000).

**Table.1. Qualitative analysis of selected parameters from plant samples**:

| Sr.No. | Chemical constituents | Test Reagents | Chirayita 1 | Chirayita 2 |
|---|---|---|---|---|
| 1 | Alkaloids | Dragendorff,s reagent | +ve | +ve |
| | | Wagner,s reagent | +ve | +ve |
| | | Mayer,s reagents | +ve | +ve |
| 2 | Carbohydrates | Molish Test | +ve | +ve |
| | | Fehling Test | +ve | +ve |
| | | Benedict Test | +ve | +ve |
| 3 | Flavonoids | Mg Ribbon and dil Hcl | +ve | +ve |
| 4 | Glycosides | NaOH Test | +ve | +ve |
| 5 | Tannins/Phenols | Ferric Chloride Test | +ve | -ve |
| | | Lieberman,s Test | +ve | +ve |

| | | | | |
|---|---|---|---|---|
| | | Lead Acetate Test | +ve | -ve |
| 6 | Protein | Xanthoproteic Test | -ve | +ve |
| | | Biuret Test | +ve | +ve |
| 7 | Starch | Iodine Test | +ve | +ve |
| 8 | Saponins | Frothing with Na HCO3 | +ve | +ve |
| 9 | Amino acids | Ninhydrin solution | +ve | +ve |

+Ve(positive), -Ve(negative

**ANTIOXIDANT ASSAY:**

Antioxidant assay confirms the in vitro antioxidant capacity of Methanolic extract is better compared with aqueous extract and had standard ascorbic acid *Chirayita* 2 has better scavenging capacity in methanolic extract as compared to aqueous extract.In aqueous extract chirayita 1 has better antioxidant activity (Schwartsmann et al.,2002; Valko et al., 2006).

**Table 2. Antioxidant activity( DPPH assay) of aqueous extract of *Chirayita*:**

| **Concentration (*μ*g/ml)** | **Ascorbic acid** | ***Chirayita* 1(%inhibition)** | ***Chirayita* 2(%inhibition)** |
|---|---|---|---|
| 50 | 73.41±1.95 | 53.36±2.17 | 50.25±0.85 |
| 40 | 70.80±2.64 | 51.2±1.22 | 49.5±0.63 |
| 30 | 56.19±3.6 | 49.93±1.20 | 47.75±0.88 |
| 20 | 43.59±4.60 | 46.49±0.58 | 42.75±0.47 |
| 10 | 30.27±7.67 | 42.06±0.96 | 40.04±0.75 |
| **IC50** | **25.83** | **35.30** | **44.89** |

**Table 3. Antioxidant activity(DPPH ASSAY) of methanolic extract of *Swerita chirayita* samples :**

| **Concentration (*µ*g/ml)** | **Ascorbic acid** | ***Chirayita* 1(%inhibition)** | ***Chirayita* 2(%inhibition)** |
|---|---|---|---|
| 50 | 71.9±0.74 | 75.38±0.89 | 65.15±0.13 |
| 40 | 68.18±1.74 | 69.19±5.89 | 59.79±0.50 |
| 30 | 55.79±3.28 | 52.80±3.33 | 48.15±0.80 |
| 20 | 46.75±1.29 | 37.7±1.35 | 42.54±0.51 |
| 10 | 35.04±5.005 | 21.36±13.20 | 36.52±0.66 |
| **IC50** | **24.18** | **28.88** | **28.28** |

**ANTIMICROBIAL ACTIVITY:**

Results showed that both samples have better antimicrobial activity with methanolic extract as compared to aqueous extract.

**Table .4 Antibacterial activity of methanol extract of *Swertia chirayita***

| **Organism** | **Extract/drug(*µ*g/ml)/zone of inhibition( mm)** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SCM 1** | | | | **SCM 2** | | | | | | **Gentamycine** | | | | | | | |
| ***E.coli*** | - | - | - | - | 10 | 20 | - | - | - | 10 | 20 | 25 | 18 | 20 | 23 | 26 | 28 | 30 |
| ***S.aureus*** | - | - | - | - | - | 1o | - | - | - | - | 10 | 20 | 13 | 18 | 21 | 25 | 26 | 33 |
| ***S.pyogns*** | - | - | - | - | 10 | 20 | - | - | - | - | - | 10 | 19 | 21 | 23 | 27 | 31 | 32 |
| ***p.aeuro-ginosa*** | - | - | - | - | - | 20 | - | - | - | - | 10 | 20 | - | - | 1 | 3 | 8 | 14 |

SCM: *Swertia chirayita* methanol extract. : No activity.

**Table 5. Antibacterial activity of Aqueous extract of *Swertia chirata*:**

| **ORGANISM:** | **Extract/drug(*µ*g/ml)/zone of inhibition( mm)** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SCA 1** | | | | | **SCA 2** | | | | | **Gentamycin** | | | | | | | |
| ***E.coli*** | - | - | - | - | - | 20 | - | - | - | - | 10 | 20 | 18 | 20 | 23 | 26 | 28 | 30 |
| ***S.aureus*** | - | - | - | - | - | 20 | - | - | - | - | - | 10 | 13 | 18 | 21 | 25 | 26 | 33 |
| ***S.pyogenes*** | - | - | - | - | - | - | - | - | - | - | - | 10 | 19 | 21 | 23 | 27 | 31 | 32 |

SCA: *Swertia Chirayita* aqueous extract. _ :No activity

## CONCLUSION

Two samples of chirayita were under study for certain physiochemical parameters of its individual constituents. These parameters include quantitative aspects. Samples were collected from raining areas of Pakistan where people tradition being used since long history. The obtained results predicted the significant aspects of our objectives particularly the targets including antioxidants, antimicrobial properties of samples. All objectives showed significant range of results those are comparable to results reported by others on similar parameters.

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