# INVESTIGATION OF PHYTOCHEMICAL CONSTITUENTS AND SOME BIOCHEMICAL PROPERTIES OF THE BARK OF *Schleichera oleosa* (Lour.) Oken. (GYO)

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## Abstract

Locally cultivated *Schleichera oleosa*(Lour.) Oken (Ceylon oak, Gyo) was chosen for the present study to investigate the phytochemicals and some biochemical properties of its bark.The preliminary phytochemical screening indicated that α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, steroids, saponins, tannins and terpenoids were present while alkaloids was absent in the bark. The physico-chemical analysis revealed that the bark contained 2.14% of ash, 9.32% of moisture, 6.25% of protein, 22.78% crudefiber, 0.83% of crude fat and58.68% of carbohydrate. The elemental analysis carried out by EDXRF method showed some elements such as Ca, K, S, Fe, Sr, Mn and Cu were present in the sample. The extractable matters (% w/w)in different polarity of solvents such as methanol, ethanol, acetone, ethyl acetate and petroleum ether were respectively observed to be 5.8, 5.0, 2.0, 1.6, 1.0 % (w/w) and water soluble matter was 3.6 % (w/w) in the sample. The ethanol extract was found to have higher total phenol content (158.67±6.76 mg GAE /g of extract) and total flavonoid content (190.0 ± 8.5 mg QE/g of extract) than the watery extract containing 148.32 ± 4.73 mg GAE/g extract of total phenol content and 143.33±7.8 mg QE/g extract of total flavonoid content. Six different extracts (PE, EA, EtOH, MeOH, $CHCl_3$ and $H_2O$)exhibited mild antimicrobial activity against some microorganisms, determined by agar well diffusion method. The antioxidant activities of ethanol extract ($IC_{50}$ = 0.56 μg/mL) and watery extracts ($IC_{50}$ = 0.69 μg/mL) determined by DPPH radical scavenging assay were higher than that of standard gallic acid significantly. Some crude extracts such as MeOH, EtOH and watery extracts exhibited the inhibition of tumor formation at the dose of 0.2 g/disc, determined on tumor producing bacteria by using Potato Crown Gall test.The ethanol extract ($LD_{50}$ = 2.69 μ g/mL) and watery extract ($LD_{50}$ = 8.85μ g/mL) also showed significant cytotoxic effect against brine shrimp (24 h).Furthermore, ethanol extract possessedhigher antiproliferative

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activity than watery extract against Hela (cervical) and MCF-7 (breast) human cancer cell lines determined by using CCK-8 Assay (Cell Counting Kit-8).

**Keywords**: *Schleicheraoleosa* (Lour.) Oken. (Gyo), total phenol content, total flavonoid content, antioxidant activity, antitumor activity, cytotoxic effect, antiproliferative activity

## Introduction

At present, there are many researches in natural products which are interesting. Plant is important and necessary to dive into life, due to the fact that it is a source of food, traditional until modern medicine. Plants are natural source of producing a wide number of bioactive chemical constituents in most efficient way and precise selectivity (Ikram *et al.*, 2009). *Schleicheraoleosa* (Lour.) Oken is a plant in the family of Sapindaceae. In Myanmar, *S. oleosa* named Gyo is found in the Central regions. It is used in the wood industry. The wood is suitable for fuel wood and charcoal, the bark is used as dye and the young leaves are eaten as vegetable. Different parts of *S. oleosa*, such as fruits, leaves, barks and seeds are used as tribal food, animal feed, seed-oil and timber. The tree also serves as important source for traditional medicines for curing pruritus, malaria, inflammation and ulcers (Bhat *et al*., 2009). Most exoticpast research has studied the properties of *S. oleosa* extracts from the bark and stems, and it was found to reduce the free radicals that cause the death of cancer cells (Thind *et al*., 2010). Antimicrobial activities were also performed against some fungal and bacterial species (Ghosh *et al*., 2011). Oil extracted from seeds can be used for the cure of itch, acne, skin burns and used as massageoil for rheumatic pains (Palanuvej and Vipunngeun, 2008). The water extract of the bark of *S. oleosa* was used to treat menstrual pain as well (Mahaptma and Sahoo, 2008).

However, there is still lack of reports from the research on locally cultivated *S. oleosa*. Hence in this study, the bark of *S. oleosa* is chosen for the investigation of some phytochemical composition and some biological activities from its ethanol and watery extracts.

## Materials and Methods

The bark samples were collected from Pyay Township, Bago Region, Myanmar. The plant was identified and authenticated at the Department of Botany, Pyay University. After collection, the washed barks were air-dried in shade for about two weeks and ground into the coarse powder with the help of a mechanical grinder. The powders of the samples were used to extract with solvents of various polarities by using ultrasonic effect and to analyze phytochemical composition.

### Phytochemical Analyses

The selected bark powders were subjected to qualitative phytochemical tests for the classification of various bioactive constituents present (Harborne, 1973). Phytochemical screenings were carried out by using standard procedures to detect the presence of alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, starch, reducing sugars and organic acids. After addition of specific reagents to the test solution, the observation of colour change or precipitate formation was noted and recorded.

### Some Physicochemical Analyses

In this study, the nutritional values such as moisture, ash, crude protein, crude fiber and crude fat of the selected bark powder was determined at Food Industries Development Supporting Laboratory (FIDSL), Myanmar Food Processors and Exporters Association (MFPEA), Yangon, Myanmar. In addition, the relative abundance of elements present in the bark sample was determined by EDXRF spectrometer at the Universities' Research Center, Yangon.

The crude extracts of the sample were prepared by extracting the sample with different solvents such as petroleum ether, ethyl acetate, ethanol, methanol and water by percolation method. All of these extracts were kept for the determination of total phenol contents, total flavonoid contents, antimicrobial activity, antioxidant activity, antitumor activity, cytotoxicity and antiproliferative activity.

**Determination of Total Phenol Contents**

The total phenol contents (TPC) in ethanol and watery extracts were estimated by the Folin-Ciocalteu method according to the procedure described by Saxena *et al.* (2013) and gallic acid was used as a standard. The sample solution (50 ppm) was prepared by dissolving 0.005 g of extract in methanol making up to 100 mL solution. Firstly, 0.5 mL of the prepared sample was mixed with 0.5 mL methanol. Then, 0.5 mL of Folin-Ciocalteu reagent (FCR: $H_2O$, 1:10) was added to the mixture and incubated for 5 min. 4 mL of 1 M sodium carbonate solution was added to each tube and the tubes were kept at room temperature for 2 h and the UV absorbance of each reaction mixture was recorded at $\lambda_{max}$ 765 nm. The control solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as mg gallic acid equivalents per g of EtOH/water extract.

**Determination of Total Flavonoid Contents**

The total flavonoids contents of the ethanol and watery extracts were measured by employing the method involving aluminium trichloride ($AlCl_3$) reagent and quercetin was used as standard(Kalita *et al.,* 2011).In the determination of total flavonoid contents, quercetin was used to construct the calibration curve. Quercetin (0.01 g) was dissolved in methanol and then diluted to various concentrations of 6.25, 12.50, 25, 50 and 100μ g/mL. A calibration curve was made by measuring the absorbance of the above different solutions at 415nm ($\lambda_{max}$ of quercetin) with a Shimadzu UV-1800 spectrophotometer. Ethanol/watery extract solution in 50 ppm was prepared by dissolving 0.005 g of each extract in 100 mL of methanol solution. Each extract stock solution (0.5 mL), 1.5 mL methanol, 0.1 mL of aluminium chloride, 0.1 mL of potassium acetate solution and 2.8 mL of distilled water were added and mixed well. The blank solution was prepared in similar way by replacing aluminium chloride with distilled water. Their absorbance was measured at 415 nm.

## Screening of Antimicrobial Activity

Antimicrobial activity of different crude extracts (PE, EA, EtOH, MeOH, $CHCl_3$ and water) of the sample was screened *in vitro* by agar well diffusion method (Perez *et al*., 1990). Bacterial cultures used in the research involved three strains of gram positive bacteria (*Bacillus subtilis, Staphyloccous aureus* and *Bacillus pumilus*), two strains of gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and one strain of fungi (*Candida albicans*). This experiment was carried out at Pharmaceutical Research Department, Insein Township, Yangon Region, Myanmar.

## Determination of Antioxidant Activity

The antioxidant activity of EtOH and $H_2O$ extracts were spectrophotometrically determined by DPPH radical scavenging assay method (Brand-Williams *et al.*, 1995). The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol with vortex mixer. The sample solution was also prepared by mixing thoroughly the 1.5 mL of 60 µM DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand for 30 min at room temperature. After 30 min, absorbance was measured at 517 nm by using a spectrophotometer UV 1601 PC (P\N 206-6750), Shimadzu corporation. Absorbance measurements were done in triplicate for each solution and the mean values obtained were used to calculate % inhibition of oxidation by the following equation,

$$\% \text{ oxidative inhibition} = \frac{A_c - (A - A_b)}{A_c} \times 100\ \%$$

% oxidative inhibition = % oxidative inhibition of test sample

$A_c$ = absorbance of the control (DPPH alone)

$A_b$ = absorbance of the blank (EtOH + Test samplesolution)

A = absorbance of test sample solution

Then $IC_{50}$ (50 % inhibitory concentration) values were also calculated by linear regressive excel program.

**Screening of Antitumor Activity**

The antitumour activity of methanol, ethanol and watery extracts of the sample was examined by Potato Discs Assay Method (Ali *et al.*, 2016). This experiment was carried out at Pharmaceutical Research Department, Insein Township, Yangon Region, Myanmar. Tumor producing bacteria, *Agrobacterium tumefaciens*, isolated from *Sandoricum koetjape* Merr. (Thitto)leaves were used in this study. This bacterial strain has been maintained as solid slants under refrigeration. For inoculation on the potato discs, 48 h broth cultures containing $5\times10^7$~$5\times10^9$ cell / mL were used. Fresh, disease free potato tubers were obtained from local markets and were transferred within 48 h to the laboratory.

Tubers of moderate sizes were surface-sterilized by immersionin 50 % sodium hypochlorite (Clorox) for 20 min. The ends were removed and soaked for 10 min more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 2.5 cm wide cork borer and 2 cm pieces were removed from each end and discarded and the remainder of the cylinder was cut into 1.0 cm thick discs with a surface-sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was dissolved in 100 mL of distilled water, autoclaved and 20 mL poured into each petri dish). Each plate contained three discs. This procedure was done in the clean bench in the sterile room. The sample (0.1, 0.15 and 0.2 g) was filtered through Millipore filters (0.22 μm) into a sterile tube. A 0.5 mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A.tumefaciens* strain (48 h culture containing $5\times10^7$~$5\times10^9$cells/mL) were added aseptically.

Controls were made in this way; 0.5 mL of DMSO and 1.5 mL of sterile distilled water were added to the tube containing 2 mL of broth culture of *A. tumefaciens* (from the same 48 h culture). Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc, surface. The process of cutting the potatoes and incubation must be conducted within 30 min. The plates were sealed with tape to minimize moisture loss and incubated at room temperature and counted with microscope and compared with control. The antitumor activity was examined by observation if tumor is produced or not.

**Determination of Cytotoxicity**

The cytotoxicity of crude ethanol and watery extracts of the sample was investigated by using brine shrimp lethality bioassay according to the procedure described by Dockery and Tomkins, 2000. The brine shrimp (*Artemiasalina*) was used in this study for cytotoxicity bioassay (Ali *et al*., 2016). Brine shrimp cysts (0.5 g) were added to the 1.5 L of artificial sea water bottle. This experiment was carried out at the Department of Chemistry, Yangon University, Myanmar. The suspension was aerated by bubbling air into the funnel and kept for 24 h at room temperature. After aeration had been removed, the suspension was kept for 1 h undisturbed, whereby the remaining unhatched eggs dropped. In order to get a higher density of larvae, one side of the separating funnel was covered with aluminium foil and the other illuminated with a lamp, whereby the phototropic larvae were gathering at the illuminated side and could be collected by pipette. The shrimp larvae were transferred to an agar well filled with 9 mL of salt water and the dead larvae counted (number N). A solution of crude extract (31.25 ~ 1000 ppm) (1 mL each) was added and the plate kept at room temperature in the dark. After 24 h, the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of ca. 0.5 mL methanol so that subsequently the total number of the animals could be determined (number G).The control solution was prepared as the above procedure by using distilled water instead of sample solution. The mortality rate M was calculated in %. Each test row was accompanied by a brine solution (number B). The mortality rate M was calculated by using the following formula:

$$M = \left[ \frac{(A - B - N)}{(G - N)} \right] \times 100$$

Where, M = percent of the dead larvae after 24 h
A = number of the dead larvae after 24 h
B = average number of the dead larvae in the brine solution after 24 h
N = number of the dead larvae before starting of the test.
G = total number of brine shrimps

### Determination of Antiproliferative Activity

Antiproliferative activity of ethanol and watery extracts were investigated in*vitro* by using cancer cell lines at Division of Natural Product Chemistry, Institute of Natural Medicine, University of Toyama, Japan. The cell lines used were Hela (human cervix cancer) and MCF 7 (human breast cancer).K562 α-Minimum essential medium with L-glutamine and phenol red (α-MEM, Wako) were used for cell cultures. All media were supplemented with 10 % fetal bovine serum (FBS, sigma) and 1 % antiobiotic antimycotic solution (Sigma). For MCF 7 cell, 1 % 0.1 M non-essential amino acid (NEAA, Gibco) and 1 % 1 mM sodium pyruvate (Gibco) were also supplemented. The *in vitro* antiproliferative activity of the crude extracts was determined by the procedure described by Win *et al.* (2015). Briefly, each cell line was seeded in 96-well plates ($2 \times 10^3$ per well) and incubated in the respective medium at 37 °C under 5 % $CO_2$ and 95 % air for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the tested samples were added. After 72 h incubation, the cells were washed with PBS and 100 μL of medium containing 10 % WST-8 cell counting kit (Dojindo; Kumamoto, Japan) solution was added to the wells. After 2 h incubation, the absorbance was measured at 450 nm. The concentrations of the crude extracts were 200, 100, 10 μg/ mL and 10, 1, 0.1 mM for positive control were prepared by serial dilution. Cell viability was calculated from the mean values of the data from three wells using the equation below and antiproliferative activity was expressed as the $IC_{50}$ (50 % inhibitory concentration) value. 5-fluorouracil (5FU) was used as positive control.

$$(\%)\ \text{Cell viability} = 100 \times \frac{\left\{\text{Abs}_{(\text{test samples})} - \text{Abs}_{(\text{blank})}\right\}}{\left\{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}\right\}}$$

## Results and Discussion

### Phytochemicals Present in the Bark of *S. oleosa*

The bark sample of *S. oleosa* collected from Pyay Township, Bago Region was found to contain some of the secondary metabolites such as steroids, glycosides, phenolic compounds and terpenoids (Table 1) according

to preliminary phytochemical screening. According to the nutritional composition of the sample (Table 2),the bark sample was found to contain higher crude fiber content (22.78 %) but lower protein content (6.25 %) and fat content(0.83 %).It was also observed to contain 2.14 % of inorganic matters (ash).

**Some Physicochemical Properties of the Bark of *S. oleosa***

As shown in Table 3, it can be seen that organic compounds are predominant in the sample, and other elements such as Ca, K and S are also present in reasonable composition but Fe and Mn were present in medium amount and Cu was present in very little amounts based on the relative abundance of elements.

The soluble matter contents of the bark powder of *S. oleosa* in solvents of different polarities were observed to be 5.8 % in methanol, 5.0 % in ethanol, 2.0 % in acetone, 1.6 % in ethyl acetate, 1.0 % in pet-ether and 3.6 % in water (Table 4). Therefore, it indicated that the phytochemicals present in bark sample were mostly the polar organic compounds.

**Table 1: Results of Phytochemical Screening of the Bark of *S. oleosa***

| Types of compounds | Extracts | Test reagents | Observation | Remark |
|---|---|---|---|---|
| Alkaloids | 1% HCl | Dragendroff's reagent<br>Mayer's reagent<br>Wagner's reagent | No orange ppt<br>No white ppt<br>No reddish brown ppt | -<br>-<br>- |
| $\alpha$-Amino acids | $H_2O$ | Ninhydrin | purple spot | + |
| Carbohydrates | $H_2O$ | 10% α -naphthol, conc: $H_2SO_4$ | red ring | + |
| Flavonoids | EtOH | Mg turning, conc: HCl | pink colour | + |
| Glycosides | $H_2O$ | 10% Lead acetate solution | White ppt | + |
| Phenolic compounds | $H_2O$ | 5% $FeCl_3$ solution | Green solution | + |
| Reducing sugars | Dil $H_2SO_4$ | Benedict's solution | Brick-red ppt | + |
| Steroids | PE | Acetic anhydride, conc: $H_2SO_4$ | Green colour | + |

| Types of compounds | Extracts | Test reagents | Observation | Remark |
|---|---|---|---|---|
| Saponins | $H_2O$ | Distilled water | Frothing | + |
| Tannins | $H_2O$ | 2% gelatin solution | white ppt | + |
| Terpenoids | $CHCl_3$ | Acetic anhydride, conc: $H_2SO_4$ | Pink colour | + |

(+) = Presence, (−) = absence

**Table 2: Approximate Nutritional Composition of the Bark of *S. oleosa***

| Nutrition Values | % (w/w, based on dry weight) |
|---|---|
| Ash content | 2.14 |
| Moisture content | 9.32 |
| Protein content | 6.25 |
| Crudefiber content | 22.78 |
| Crude fat content | 0.83 |
| Carbohydrate content | 58.68 |

**Table 3: Relative Abundance of Elements in the Bark of *S.oleosa* by EDXRF Spectrometry**

| Macro and Micronutrients | Relative aundance(%) |
|---|---|
| Ca | 4.714 |
| K | 0.472 |
| S | 0.177 |
| Fe | 0.091 |
| Sr | 0.019 |
| Mn | 0.004 |
| Cu | 0.001 |
| COH | 94.521 |

**Table 4: Extractable Matter Contents in the Bark of *S.oleosa***

| Solvents | Weight of extractable matter (% w/w) |
|---|---|
| Water | 3.6 |
| Methanol | 5.8 |
| Ethanol | 5.0 |
| Acetone | 2.0 |
| Ethylacetate | 1.6 |
| Petroleum Ether | 1.0 |

**Total Phenol and Total Flavonoid Contents in the Bark of *S. oleosa***

Total phenol and total flavonoid contents in the bark of *S. oleosa* are shown in Figure 1 and Table 5. Total phenol contents of the extracts were calculated from the regression equation of calibration curve (Y = 0.0638 x + 0.0169; $R^2$ = 0.9941) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight. To perform the calculation of the total flavonoid contents in the samples by using Kiranmai *et al.* (2011) method, a standard curve is needed which is obtained from a series of absorbance of different quercetin concentrations (Y = 0.0019x +0.0115; $R^2$ = 0.9955). It can be seen that the total phenol of the ethanol extract (158.67±6.76 mg GAE/g of extract) was slightly higher than that of watery extract (148.32 ± 4.73 mg GAE/g of extract. In the case of total flavonoid content, total phenol content of ethanol extract (190 ± 8.5 mg QE/g of extract) is significantly higher than that of water extract (143.33 ± 7.8 mg QE/g of extract). The ethanol extract showed higher total phenol content and total flavonoid content than water extract, indicating that phenolic and flavonoids compounds were more soluble in organic solvent than water. Generally, extracts with a high amount of phenolic compounds might exhibit high antioxidant activity.

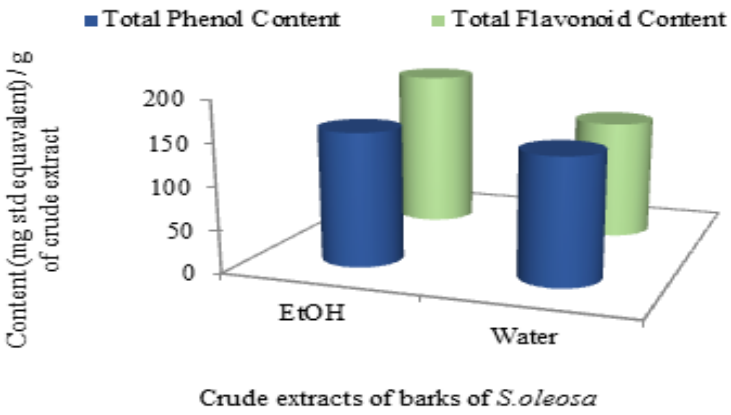


**Figure 1**: Total phenol and total flavonoid contents of *S. oleosa* Bark

**Table 5: Total Phenol Content (TPC) and Total Flavonoid Content (TFC) of Crude Extracts**

| Types of compounds | EtOH extract | Water extract |
|---|---|---|
| TPC (mg GAE ± SD)/g of extract | 158.67±6.76 | 148.32±4.73 |
| TFC (mg QE ± SD)/g of extract | 190.00 ±8.5 | 143.33±7.8 |

**Antioxidant Activity of the Bark of *S. oleosa***

Most of the medicinal plants possess phytochemicals and antioxidant activity. Flavonoids and tannins are phenolics which are a major group of compound in plants. These compounds act as primary antioxidant or free radical scavengers (Ayoola*et al*., 2008). The antioxidant activity of watery and ethanol extracts of the sample was studied by DPPH free radical scavenging assay (Marinova and Batchvarov, 2011). Gallic acid was used as standard. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is widely used to investigate the free radical scavenging activities of several natural compounds such as crude extracts of plants. DPPH radical is scavenged by antioxidant through the donation of electron forming the reduced DPPH. Sample's colour change from purple to pale yellow which can be quantified by its decrease of absorbance at wavelength 517 nm (Maw *et al*., 2011). The radical scavenging activity of crude extracts were expressed in term of % RSA and $IC_{50}$ (50% inhibition concentration). The results are shown in Table 6. From these observations, the larger DPPH radical scavenging activity was observed in ethanol extract, which inhibited 50% of free radicals at the concentration of 0.56 μg/mL ($IC_{50}$) than the water extract which inhibited 50% of free radicals at the concentration of 0.69 μg/mL ($IC_{50}$). It can be inferred that the antioxidant potency of the ethanol extract was found to be stronger than that of the watery extract, and both extracts were observed to be significantly higher than standard gallic acid ($IC_{50}$ = 5.07 μg/mL) in antioxidant property.

**Table 6: DPPH Free Radical Scavenging Activity (%RSA) and $IC_{50}$ of Crude Extracts of the Bark Sample and Standard Gallic Acid**

| Samples | % RSA ± SD at Different Concentrations (μg/mL) | | | | | | $IC_{50}$ (μg/mL) |
|---|---|---|---|---|---|---|---|
| | 0.3125 | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| EtOH extract | 22.08 ± 0.38 | 57.62 ± 1.75 | 58.94 ± 1.75 | 61.59 ± 5.17 | 70.42 ± 4.69 | 80.13 ± 0.66 | 0.56 |
| Water extract | 43.49 ± 5.63 | 46.14 ± 1.38 | 82.29 ± 0.86 | 84.37 ± 2.65 | 85.46 ± 3.23 | 86.67 ± 3.09 | 0.69 |
| Standard Gallic Acid | 27.06 ± 13.01 | 33.12 ± 5.15 | 34.42 ± 10.45 | 41.13 ± 3.33 | 49.35 ± 1.12 | 97.40 ± 0.65 | 5.07 |

## Antimicrobial Activity of the Bark of *S. oleosa*

The antimicrobial activity was assessed by agar well diffusion method which is equally suited to the screening of antibiotics or the products of plant evaluation and is highly effective for rapidly growing microorganisms and the activities of the test extracts are expressed by measuring the zones (mm) of inhibition. Generally, the more susceptible the organism, the bigger is the zone of inhibition. Antimicrobial activities of six different extracts (PE, EA, EtOH, MeOH, $CHCl_3$, $H_2O$) of the sample were also determined against some fungal and bacterial species. The observed data are tabulated in Table 7. Generally, ethyl acetate and ethanol extracts have medium activities on the gram positive bacteria: *B. subtilis*and *B. pumilus* and low activity against two gram negative bacteria *S. aureus*and *P. aeruginosa*. Although ethyl acetate extract showed medium activity on *C. albicans*, ethanol extract did not inhibit well. Ethanol extract was found to possess medium antimicrobial activity against *E. coli*,however, ethyl acetate exhibited low activity. Methanol, chloroform and water extracts were observed to exhibit low antimicrobial activities against all tested microorganisms whereas pet-ether extract showed low activity against only *B. subtilis*, *P. aeruginosa*, *C. albicans* and *E. coli*.

**Table 7: Inhibition Zone Diameters of some Crude Extracts against Six-Microorganisms by Agar Well Diffusion Method**

| Organisms | Inhibition Zone Diameters (mm) of Crude Extracts | | | | | | |
|---|---|---|---|---|---|---|---|
| | **PE** | **EA** | **EtOH** | **MeOH** | **$CHCl_3$** | **Water** | **Control** |
| *B. subtilis* | 11 (+) | 16(++) | 15 (++) | 14 (+) | 12(+) | 12(+) | - |
| *S. aureus* | - | 13 (+) | 13 (+) | 14(+) | 12(+) | 12(+) | - |
| *P. aeruginosa* | 11(+) | 11(+) | 14(+) | 12(+) | 12(+) | 13(+) | - |
| *B. pumilus* | - | 16(++) | 15(++) | 12(+) | 12(+) | 12(+) | - |
| *C. albicans* | 11(+) | 15(++) | 14(+) | 12(+) | 12(+) | 13(+) | - |
| *E. coli* | 11(+) | 13(+) | 15(++) | 11(+) | 11(+) | 13(+) | - |

Agar Well – 10 mm ; control - solvent used; 10 mm ~ 14 mm (+) (low activity); 15 mm ~ 19 mm (++) (medium activity) ; above 20 (+++) (high activity)

## Antitumor Activity of the Bark of *S. oleosa*

The antitumor activity of ethanol and watery extracts of the bark sample was investigated by using PCG test with the isolated tumor forming bacterium *A. tumefaciens*. The 48 h broth cultures containing 5 x $10^9$ cells/mL were used to inoculatethe potato disc. The tested samples were dissolved in DMSO to dilute and the diluted samples were mixed with the bacterial culture for inoculation. After preparing the inoculums, the bacterial suspension was inoculated on the cleaned and sterilized potato discs, and incubated at room temperature for 3 days. Then the tumors appeared on potato discs and checked by staining the knob with Lugol's ($I_2$-KI) solution. In the control, the formation of white knob on the blue background indicated tumor formation. The active test samples did not form any tumor on the potato discs and its surface remained blue. This experiment revealed that all of the tested samples exhibited the inhibition of tumor formation at the dose of 0.2 g/disc after 5 days and 7 days treatment (Table 8).

**Table 8: Observation of Tumor Inhibition by Different Concentrations of Crude Extracts of the Bark Sample**

| Extracts | Days | Observation of Tumor Formation by Different Concentrations of Crude Extracts | | |
|---|---|---|---|---|
| | | 0.10 g/disc | 0.15 g/disc | 0.20 g/disc |
| MeOH | | + | + | - |
| EtOH | 5 | + | + | - |
| $H_2O$ | | + | + | - |
| MeOH | | + | + | - |
| EtOH | **7** | + | + | - |
| $H_2O$ | | + | + | - |
| Control | ++ | | | |

(+) Tumor formation (-) No Tumor formation

## Cytotoxicity of the Bark of *S. oleosa*

The cytotoxicity of water and ethanol extracts fromthe sample was evaluated by brine shrimp cytotoxicity bioassay. This bioassay is general toxicity screening for bioactive phytoconstituents and their derivatives. A model animal that has been used for this purpose is the brine shrimp, *Artemiasalina* (Tawaha, 2006). The cytotoxicity of crude extracts was expressed in term of mean ± SEM (standard error mean) and $LD_{50}$ (50% Lethality Dose) and the results are shown in Table 9. In this experiment, standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is well-known toxic in this assay (Salinas and Fernendez, 2006) and caffeine is a natural product. As shown in Table 9, the ethanol extract of the selected sample was more toxic to brine shrimp than the watery extract. The $LD_{50}$ values of EtOH and watery extracts were 2.69 µg/mL and 8.85 µg/mL, respectively. On the other hand, the ethanol and watery extracts were less toxic than standard $K_2Cr_2O_7$($LD_{50}$ 1.5 µg/mL) and more toxic than caffeine($LD_{50}$ >1000 µg/mL) to brine shrimp. These results revealed that the plant extracts possessed cytotoxic activity and can be used as a source of cytotoxic compounds. The selected plant sample can be used in traditional medicine to treat many kinds of diseases. The reported cytotoxic plants in this study are worth of further pharmacological and phytochemical studies in order

to define what kind of bioactivity they have and to isolate the natural active constituents, which are responsible for the activity.

**Table 9: Cytotoxicity of Ethanol and Watery Crude Extracts of the Bark Sample**

| Crude extract | Dead % by using different concentrations (μg/mL) of samples | | | | $LD_{50}$ (μg/mL) |
|---|---|---|---|---|---|
| | 1 | 10 | 100 | 1000 | |
| EtOH | 44.7±1.7 | 73.1±1.1 | 85.5±7.8 | 87.9±7.4 | 2.69 |
| Water | 36.4±2.7 | 51.9±8.7 | 77.6±2.5 | 80.5±6.4 | 8.85 |
| *$K_2Cr_2O_7$ | 48.63±19.19 | 73.13±4.076 | 74.67±11.8 | 100±0 | 1.50 |
| *Caffeine | 0 ± 0 | 0 ± 0 | 9.582±0.917 | 12.73±4.103 | >1000 |

*****standard

## Antiproliferative Activity of the Bark of *S. oleosa*

Cancer is a malignant tumor or malignant neoplasm, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Many traditional plant treatments for cancer are used throughout the world, and some of these plants have been scrutinized while a good number of them are yet to receive scientific scrutiny. Among them, the bark of *S. oleosa* was selected for this study since they are widely distributed in Myanmar. Antiproliferative activity is the activity relating to a substance used to prevent or retard the spread of cells, especially malignant cells, into surrounding tissues. Antiproliferative activity was studied in vitro using human cancer cell lines.Screening of antiproliferative activities of ethanol and watery extracts from the bark of *S. oleosa*was done by using two human cancer cell lines such as Hela (human cervix cancer) and MCF7 (human breast cancer). Antiproliferative activity was expressed as the $IC_{50}$ (50 % inhibitory concentration) value. 5-Fluorouracil was used as positive control. The antiproliferative activity of crude extracts are summarized in Table 10. From the results, the $IC_{50}$ values were found to be 142.58 μg/mL againstcervix cancer and 135.63 μg/mL against breast cancer by ethanol extract and 150.31 μg/mL against cervix cancer and 178.18 μg/mL against

breast cancer by watery extract, respectively. Since the lower the $IC_{50}$ values, the higher the antiproliferative activity, ethanol extract was more potent than watery extract in antiproliferative activity on the selected cell lines.

**Table 10: Antiproliferative Activity of Crude Extracts against Two Types of Cancer Cell Lines**

| Samples | $IC_{50}$ (μg/mL) of various samples against tested cell lines | |
|---|---|---|
| | **Hela** | **MCF 7** |
| EtOH Extract | 142.58 | 135.63 |
| Water Extract | 150.31 | 178.18 |
| 5FU* | 3.102 | 4.821 |

Hela - human cervical cancer cell lines
MCF 7 - human breast cell lines
5FU* - Fluorouracil (drug for cancer)

## Conclusion

From the overall assessment concerning with investigation of phytochemicals and some biological activities on the bark of*S. oleosa* (Lour.) Oken. (Gyo), ethanol extract was observed to possess higher antimicrobial, antioxidant and antiproliferative activities than watery extract, it might be due to its higher contents of total phenols and total flavonoids. According to the observations, since the ethanol and watery extract of Gyo bark showed these activities, these extracts may be effectively used for the treatment of skin disease, wound infections, diarrhea and also as antioxidant for curing the oxidative stress related diseases, some forms of cancer and some age-related disorders. The quantitative and qualitative phytochemical analytical data are also expected to be applicable to some extent in the medicinal formulation by using the bark of *S. oleosa*.

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## References

Ali, N., Zada, A., Ali, M. and Hussain, Z. (2016).“Isolation and identification of *Agrobacterium tumefaciens* from the Galls of Peach Tree”.*Journal of Rural Development and Agriculture*, vol. 1(1), pp. 39-48.

Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Bello, A. A. A., Obaweya, K., Ezennia, E. C., and Atangbayila, T. O. (2008). “Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria”.*Tropical Journal of Pharmaceutical Research*, vol. 7, pp. 1019–1024.

Bhat, S. V., Nagasampagi, B. A. and Meenakshi, S. (2009). *Natural Products:Chemistry and Applications*. Oxford: 1st Ed., Alpha Science International Ltd., 1-242.

Brand-Williams, W., Cuvelier, M. E.andBerse, C. (1995). "Use of a Free Radical Method to Evaluate Antioxidant Activity".*Lebensm.-Wiss. u.-Technol*., vol. 28 (1),pp. 25-30.

Dockery, M. and Tomkins, S. (2000). *Brine Shrimp Ecology*.London : 1st Ed., The British Ecology Society, pp. 92-93.

Ghosh, P., Chakraborty, P., Mandal, A., Rasul, M. G., Chakraborty, M. and Saha, A.(2011). “Triterpenoids from Schleicheraoleosa of Darjeeling Foothills and their antimicrobial activity”.*Indian Journal of Pharmaceutical Science*, vol.73, pp. 231-233.

Harborne, J. B. (1973). *Phytochemical Methods : A Guide to Modern Techniques of Plant Analysis*.London: 2nd Ed., pp. 37-38.

Ikram, E. H. K., Eng, K. H., Jalil, A. M. M., Ismail, A., Idris, S., Azlan, A., Nazri, H. S. M. , Diton, N. A. M. and Mokhtar, R. A. M. (2009). “Antioxidant capacity and total phenolic content of Malaysian underutilized fruits”. *Journal of Food Composition and Analysis*,vol. 22, pp. 388-393.

Kalita, S., Kumar, G., Karthik, L. and Rao, K. V. B. (2011)."Phytochemical Composition and *In Vitro* Hemolytic Activity of *Lantana camara* L.(Verbenaceae) Leaves".*Pharmacology*,vol.1, pp. 59-67.

Kiranmai, M., Mahender, K. C. B., and Ibrahim, M. D. (2011). “Free Radical Scavenging Activity of Neem Tree (*Azadirachta indica* A. Juss Var., Meliaceae) Root Bark Extract”.*Asian Journal of Pharmaceutical and Clinical Research*,vol.4 (4), pp.134-136.

Mahaptma, S. P. and Sahoo, H.P. (2008). “An Ethano-medico-botanical Study of Bolangi, Orissa, India: Native plant remedies against Gynaecological diseases”. *International Journal of Ethanobotanical Research*, vol. 12, pp. 846-854.

Marinova, G. and Batchvarov, V.(2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH", *Bulgarian Journal of Agricultural Science*.vol.17, pp. 11–24.

Maw, S. S., Mon, M. M. and Oo, Z. K. (2011)."Study on Antioxidant Activities of Some Herbal Extracts".*World Academy of Science, Engineering and Technology,* vol.51, pp. 450–452.

Palanuvej, C. and Vipunngeun, N. (2008). "Fatty Acid Constituents of Schleicheraoleosa(Lour) Oken. Seed Oil".*Journal of Health Research*,vol. 22(4), pp. 203.

Perez, C., Paul, M. and Bazerque, P. (1990). "Antibiotic Assay by Agar Well Diffusion Method.". *Alta BioMed Group Experiences,*vol. 15, pp.113-115.

Salinas, M. M. G. and Fernandez, S. S. (2006). "A Modified Microplate Cytotoxicity Assay with Brine Shrimp Larvae (*Artemiasalina*)".*Pharmacology*, vol.3, pp. 633–638.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013)."Phytochemistry of Medicinal Plants".*Journal of Pharmacognosy and Phytochemistry*,vol. 1(6), pp.168-172.

Tawaha, K. A. (2006). "Cytotoxicity Evaluation of Jordanian Wild Plants Using Brine Shrimp Lethality Test". *J. Appl. Sci*., vol. 8, pp. 12–17.

Thind, T. S., Rampal, G., Agrawal, S. K., Saxena, A. K., and Arora, S. (2010). "Diminution of Free Radical Induced DNA Damage by Extracts/Fractions from bark of *Schleicheraoleosa* (Lour.) Oken". *Drug and Chemical Toxicology*, vol. 33, pp. 329-336.

Win, N. N., Ito T., Aimaiti, S., Kodama, T., Imagawa, H., Ngwe, H., Asakawa, Y., Abe, I., Morita, H. (2015). "Kaempulchraols I-O: New Isopimarane Diterpenoids from *Kaempferiapulchra* Rhizomes Collected in Myanmar and their Antiproliferative Activity". *Tetrahedron*, vol. 71, pp. 4707-4713.