

PHYTOCHEMICAL AND BIOLOGICAL STUDY OF ALEURITOPTERIS ANCEPS

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ABSTRACT

This study was carried out to investigate phytochemical and biological study of *Aleuritopteris anceps*, also known as Rani sinka. The whole plant was collected from Kathmandu and Lalitpur district about 1300 m altitude in June-July and subjected to hexane, ethyl acetate and methanol extraction by maceration process. The phytochemical study revealed the presence of alkaloids, saponins, glycosides, terpenoids, tannins, carbohydrates and phenols. The biological study was done for antioxidant (by DPPH scavenging method), antimicrobial sensitivity test (by cup plate method) and anti-diabetic activity (by oral glucose tolerance test in glucose loaded rats). Antioxidant activity was

considerable with IC₅₀ value 29.22 µg/ml, 147.48 µg/ml and 453.67 µg/ml methanol, ethyl acetate and hexane respectively. Antimicrobial activity was considerable to Gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus* Gram negative bacteria *Salmonella typhi* but not at the concentrations of 100 mg/ml against *Escherichia coli*. The hypoglycemic effect of 200 mg/kg and 400mg/kg methanol extract was found to be 26.23% and 26.79% respectively where as standard drug Glimeperide showed 32.52%. This study concludes that it could serve as a source of potent antioxidant, antimicrobial and anti-diabetic agents. Therefore this study can be basis for the further research to find out more detail information regarding the relationship between antioxidant activity and other phytochemical content which may help to highlight the chemicals which are responsible for this activity.

KEYWORDS: *Aleuritopteris anceps*, Rani sinka, Antioxidant activity, Antimicrobial activity, Anti- diabetic activity.

INTRODUCTION

The study of medicinal plant is based on different medicinal systems such as Ayurveda, Unani and Siddha. It is reported that, in Nepal, traditional healers use 1614 plant species of medicine.^[1] Plant plays an important role in traditional systems to manage various types of diseases like respiratory tract infection, gastrointestinal problems, dermatological disorders, hepatic problems and cardiovascular disorder.^[2]

According to census 2001, the country contains 100 caste/ethnic groups and 92 languages. Among them 59 ethnic groups are documented as indigenous nationalities. People are living in different geographical belts depends on local plant and plant products to meet their daily requirements intended for food, fodder, medicines etc. Such type of ethno botanical knowledge on various plants developed by human beings by their self-experience, trial and accidents is now in the state of wearing down and is decreasing day by day because of growing modernization and urbanization.^[3]

Most of the beneficial effects of plant material results from the combination of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, tannins, phenols, sterols, flavonoids, resins fatty acids gums which are capable of producing definite physiological action on body.^[4] The knowledge of the traditional healing practices, using wild plants, is now fast disappearing due to modernization and the tendency to discard their traditional lifestyle and gradual migration to main stream. There is an urgent need and documentation of valuable knowledge for wealth. According to WHO, more than 80% of the world's people rely their primary healthcare on herbal medicine, most of which are remedies made from plants.^[5]

Medicinal plants have an important role in the socio-cultural and health care need of people of the emerging and developing countries so there is need of proper utilization of medicinal plants to develop value-added product is important like our country Nepal. This framework will lay a strong foundation for the future development of herbal medicines in the health care system. Drug resistance to human pathogenic bacteria and fungus infections has further complicated in the treatment of various infectious disease. Search for newer drugs from plant has been increasing day by day due to emergence of new diseases and alarming side-effects

of synthetic drugs. Thus, the present study will be helpful in identifying the antimicrobial potential of selected plant candidate for the best alternative to the indiscreet use of current synthetic antibiotics.

Different types of reactive oxygen species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and free radicals, such as the hydroxyl radical (OH) and superoxide anion (O_2^-) are produced as normal products of cellular metabolism. The rapid production of such free radicals in the body lead to oxidative damage to biomolecules and may cause various disorders such as cancer, cardiovascular disease, diabetes, inflammatory diseases, asthma, neurodegenerative diseases and premature aging.^[6]

Recently, Antioxidant from natural sources has been increased for use in food, cosmetic and pharmaceutical products to replace synthetic antioxidants, which are being restricted due to their carcinogenicity.^[7] The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous.

Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity. Flavonoids are an important class of phenolic compounds and have potent antioxidant activity. It is reported that, Flavonoidic derivatives have exposed a broad series of antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities.^[8] Thus, the current study is focused on determination of total phenolic and flavonoid content and their influence on antioxidant activity, antimicrobial activity and anti-diabetic activity.

Aleuritopteris anceps is one of the well-known traditional medicinal plants, widely distributed in middle hilly region of Nepal. Thus, this study is a combinatorial approach to find potential bioactive compound. This study will help to explore the medicinal potential of the herb being focused on variations of chemical constituents, biological activity and give way for further exploration on this plant in Nepal.

MATERIALS AND METHOD

Study Design

Experimental and descriptive research design.

Plant Material

Whole plant was collected from Kathmandu and Lalitpure district about 1300m altitude in June-July in 2014 and then it was identified as *Aleuritopteris anceps* in National Herbarium Center and Plant laboratory, Godavari, Nepal.

Chemicals and apparatus

Chemicals used were hexane, ethyl acetate, methanol, diphenyl-1-pyrylhydrazyl reagent (Hi-Media), ascorbic acid, Ofloxacin, Glimepiride and Apparatus used were UV-Visible spectrophotometer (SHIMAZU) and Rotary shaker (Associated Scientific Technologies Delhi, India).

Organisms

Pure bacterial culture of two Gram positive organisms *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* and two Gram negative organisms *Salmonella typhi* and *E.coli* (ATCC 25922) were obtained from the Microbiology Laboratory of National College, NIST.

Plant processing

Whole plant material was washed thoroughly with water and shade dried at room temperature. Dried sample was crushed by grinder as to make a coarse powder. The total of 200 g of powder material was extracted with 2500ml of non-polar to polar solvents (hexane, ethyl acetate and methanol) maceration process occasionally shaking by using Rotary shaker for 7 days and the extract was dried in room temperature and concentrated product was collected as final extract of plant then it was preserved at refrigerator at 4⁰C for further analysis.

Phytochemical Screening

The phytochemical screening (qualitative) was done to identify the main group of chemical constituents present in different extract of *Aleuritopteris anceps* by their color reactions with different reagents.^[9;10] Each extract was subjected for alkaloids, terpenoids, saponins, glycosides, carbohydrates, phenols, flavonoids and tannins.

Determination of Total Phenolic Content

Procedure

Total phenolic content in extract was determined with Folin-Ciocalteu (FC) reagent using gallic acid as a standard phenolic compound.^[11] Accurately 0.5 ml of each extract (5mg/ml)

was separately mixed with Folin-Ciocalteu (FC) reagent (5 ml, 1:10 v/v diluted with distilled water) and aqueous sodium carbonate (Na_2CO_3 , 4 ml, 1M) solution. The mixture was kept to stand for 15 minutes at room temperature. Absorbance of reaction mixture was recorded at 765 nm using spectrophotometer. Stock solution of galic acid (10 to 80 $\mu\text{g}/\text{ml}$) was used to constructing the standard curve and the total phenolic compound concentration in the extract was expressed as milligram of galic acid equivalent per gram of dry weight (mg GAE/g) of the extract.

Total Flavonoid Content determination

Procedure

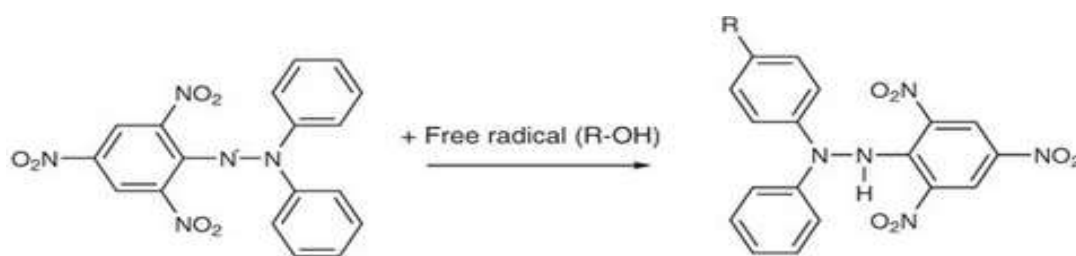
Total flavonoid content in extract was determined according to colorimetric method.^[11] Briefly 0.5ml of each extract (50mg/ml) was separately mixed with 1.5 ml methanol, 0.1 Aluminium trichloride (AlCl_3 , 10%). Subsequently 0.1 ml of 1M potassium acetate and 2.8 ml distilled water was added to each test tube and the reaction mixture was allowed to stand for 30 minutes. Then the absorbance was measured in 415nm with UV-visible spectrophotometer. Quercetin was used for constructing the standard curve (10 to 50 $\mu\text{g}/\text{ml}$) and total flavonoid compounds concentration in the extracts was expressed as milligram of Quercetin equivalent per gram of dry weight (mg QE/g) of extract.

Biological study

The plant extract were investigated for antioxidant activity, antimicrobial susceptibility and anti-diabetic activity.

Antioxidant Activity by DPPH scavenging

The DPPH assay is based on the capacity of an antioxidant to donate a hydrogen radical an electron to DPPH radical, which is stable free radical with deep violet color. When an odd electron become paired in the presence of free radical get reduced to corresponding hydrazine, DPPH-H form and the solution gets decolorized from its initial deep violet to light yellow color. The degree of fall in the absorbance is measured spectrophotometric and is proportional to the concentration of the antioxidant.^[12; 13]

**Diphenylpicrylhydrazyl (free radical)****Diphenylpicrylhydrazine (non-radical)****Procedure**

The antioxidant activity of the extract was measured on the basis of the scavenging activity of the stable 1,1-diphenyl 2-picrylhydrazyl (DPPH) free but stable at room temperature stock solution of DPPH and test samples of the extract was made at various concentrations (5, 20, 40, 60, 80 and 100 µg/ml). Similarly reference samples of ascorbic acid were made at similar concentration. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations. Corresponding blank sample was prepared by mixing of 1ml methanol and 1ml DPPH solution was used as control. DPPH was mixed with the solvent used in preparation of test solution and kept in dark for 30 minutes. Similarly, DPPH was mixed with different concentration test sample and ascorbic acid and kept in dark. The absorbance was measured at 517nm by spectrophotometer and % scavenging was calculated by the equation.^[14]

$$\text{Scavenging \%} = \frac{A_c - A_s}{A_c} \times 100.$$

Where

A_c is the absorbance of the control.

A_s is the absorbance of the sample

The inhibition concentration (IC_{50}) value was determined by extrapolating the graph of % scavenging activity versus the concentrations on the basis of the scavenging results of the amount of antioxidant required to reduce the initial radical concentrations analysis IC_{50} is defined as the amount of antioxidant required to reduce the initial radical concentration by 50%. Lower the IC_{50} value higher was the antioxidant effects of the extract.^[15]

Antimicrobial Screening of the Extracts**Introduction**

Medicinal plants represent a rich resource of antimicrobial agents. Many plants are used as medicinally in different countries as a good source of many potent and powerful drugs.^[16] A

large no of medicinal plants parts is used for extract as raw drugs and supply in the market as the raw material for many herbal industries.^[17] Every antimicrobial drug comes with an active period beyond which it gets resistance to bacteria. Bacterial from clinical and non-clinical setting are becoming ever more resistance to usual antibiotics.^[18]

Resistance to antimicrobials is the one of the greatest threat become a major human health concern globally. Thus alarming is increasing to discover new antimicrobial agent in the pharmaceutical channel for the replacement of presently existing antimicrobials.^[19]

Anti-microbial activity was performed in three extracts of plants viz. hexane, ethyl acetate and methanol of concentration of 100mg/ml by dissolving in DMSO.

Reference standard: 50µg/ml Ofloxacin.

Cup plate Method: Antimicrobial screening of the extract

Preliminary antimicrobial test of *Aleuritopteris anceps* extract was carried out by cup plate method. The extract, which was show antimicrobial activity, then was to be subjected for antimicrobial screening using reference standard by tube dilution method for determination of minimum inhibitory concentration and minimum bactericidal concentration. Cup plate technique according was used in antibiotic assay with modification. Muller-Hinton agar plates of 4mm thickness were prepared and cup of uniform diameter of 6mm were bored. Antimicrobial screening of different extract were dissolved in 10% dimethyl sulfphoxide(DMSO) was done at 100mg/ml and DMSO as control against two Gram positive organism and two Gram negative organism by inoculating bacterial broth of 0.5 McFarland standard turbidity in Muller-Hinton agar. Then the plates were incubated in at 37⁰C and zone of inhibition were measured after 24 hours.^[20,21]

Procedure

1. Muller-Hinton Agar was prepared according to the method describe in pack. The media was sterilized in autoclave at 121 for 20 minutes. Then the sterilized media was cooled to about 50⁰C then poured to sterilized Petri dish with the size of 90 mm diameter and left for solidification.
2. Bacterial suspension was prepared in Muller-Hinton broth to prepare broth equivalent to turbidity of 0.5 McFarland standards.
3. The suspension was then swabbed on the media with sterile cotton swab.

4. With the help of sterile cork borer having the diameter of 6 mm, cups were made in agar plates and labeled appropriately with the help of permanent marker pen.
5. Different extract (hexane, ethyl acetate and methanol) was dissolved in 10% dimethyl sulphoxide (DMSO) solution.
6. 50µl of concentration (100mg/ml) of extract and vehicle was placed in each cup of medium. Similarly standard Ofloxacin (50µl) was placed.
7. All the plates were then incubated at 37⁰C for 24 hrs.
8. After 24 hrs, the zone of inhibition was measured and MIC value has carried out.

Determination of MIC value

The Minimum inhibitory concentrations which inhibit the visible growth of a microorganism's were determined by the micro broth dilution method. The MIC was performed as on described below on extracts that showed efficacy against microorganism by the cup plate method (Inhibition zone is half of the positive control). Dilution susceptibility testing method is used to determine the minimal concentration of antimicrobial needed to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in methanol extracts in nutrient broth media.

Procedure

- The minimum inhibitory concentration (MIC) was determined by micro dilution method using serially diluted plant extracts.
- Different 11 test tubes were taken and 1ml of extracts were diluted to get series of concentrations from 100mg/ml in sterilizes 1ml nutrient broth and the microorganism suspension of 50 µl was added to the broth dilutions
- 1ml nutrient broth and standard Ofloxacin 50µl was placed in positive control test tube similarly 1ml nutrient broth and 50µl DMSO was placed as negative control test tube.
- These all test tube were incubated for 24 hours at 37⁰C. MIC of each extract was taken at the lowest concentration that did not give any visible bacterial growth.

Anti-diabetic effects of plant extract on rats

Diabetes is a metabolic disorder due to impaired secretion or diminished effect of endogenous insulin. Most of oral hypoglycemic agents cause side effects. About 285 million people are affected in 2010 and it is estimated that it will be increase about 439 million by 2030. It is estimated that it will be increase 69% in developing country and 20% in developed

country in between 2010 and 2030.^[22] It is reported that among two diabetes mellitus type 1 and type 2, type 2 DM is the commonest 90%-95% of the total diabetic population. A study has reported that people diagnosed with type 2 is increasing at a rate of 4%-5% per year globally.^[23] Many oral hypoglycemic drugs have typical profile of adverse effects.^[24] Many plants are used in folk medicine new oral hypoglycemic agents from medicinal plant may be useful source for development of new drug molecule to adapt current remedies.^[25]

Oral glucose tolerance test^[26; 27; 28; 29]

Oral glucose tolerance test is simple method to measured body's ability to use or remove excess sugar from blood and also used to hypoglycemic properties of extracts. Fasting greatly decrease the rate of disappearance of glucose from the blood after hyperglycemia, while in non-fasting condition, tolerance is very high. So, extracts is given to overnight fasted (16-18hrs) rats orally or intra peritoneal to measure tolerance. Then, a load of glucose is loaded orally and their blood glucose level is determined. The tolerance of rat to glucose gives indication of hypoglycemic activity.

Procedure

- Albino Westar rats were fasted for 16-18 hours with distil water.
- The fasted rats were divided into 4 groups 3 rats in each.
- Initial blood glucose level of each rats were measured by tail-tip method using glucometer.
- Standard group I: Glimepiride 0.43mg/kg orally made in distilled water.
- Test group II: Methanol extract orally at 200mg/kg dissolved in distilled water.
- Test group III: Methanol extract orally at 400mg/kg dissolved in distilled water
- Control group IV: Distilled water orally.
- After 30 minutes, the blood glucose levels were again monitored and the rats were loaded with 3gm/kg glucose made in 10 gram in 50 ml distilled water.
- The blood glucose levels of rats were measured at 30, 60, 120 and 180 minutes after the glucose load.

The percentage glycemic effect was then calculated as follows:

Percentage lowering blood level= $(1 - W_e / W_c) \times 100$.

Where

W_e is blood glucose level of test/standard

W_c is blood glucose level of control

RESULTS

Extractive value

The yield percentage for each extract in maceration method was calculated and the yield of different three extract was found to be high in methanol.

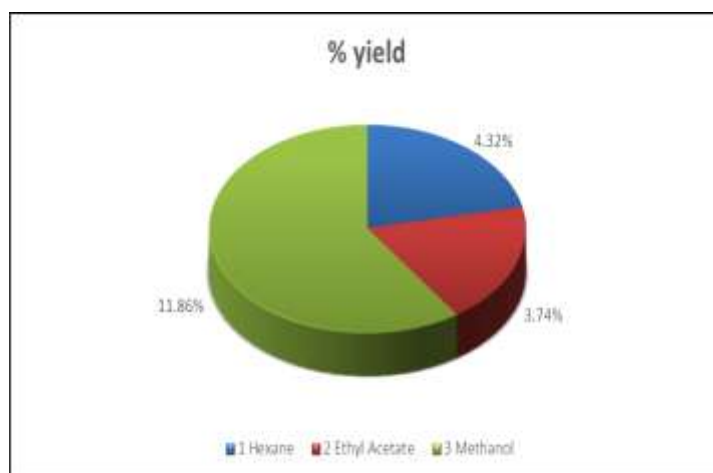


Figure 1: Extractive value of *Aleuritopteris anceps*.

Phytochemical Screening

Phytochemical screening included qualitative test by color reaction and quantitative estimation of total phenol and flavonoid content of different extracts of *Aleuritopteris anceps*.

Qualitative method of phytochemical screening

Phytochemical screening of the plant showed the presence of different group of active constituents in three different extracts. The result obtained is tabulated as follows:

Table No 1: Phytochemical screening of different extract of *Aleuritopteris anceps*.

| S.N | Phytoconstituents | Hexane | Ethyl Acetate | Methanol |
|-----|-------------------|--------|---------------|----------|
| 1 | Alkaloids | + | + | + |
| 2 | Saponins | - | + | + |
| 3 | Glycosides | + | + | + |
| 4 | Terpenoids | + | + | + |
| 5 | Flavonoids | + | + | + |
| 6 | Tannins | + | + | + |
| 7 | Carbohydrates | + | + | + |
| 8 | Phenols | + | + | + |
| 9 | Protein | - | - | - |

Present (+) and absent (-), different extracts.

Quantitative Phytochemical Screening

Total Phenolic Content determination

Calibration curve of standard gallic acid was obtained by Microsoft Excel 2007 where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

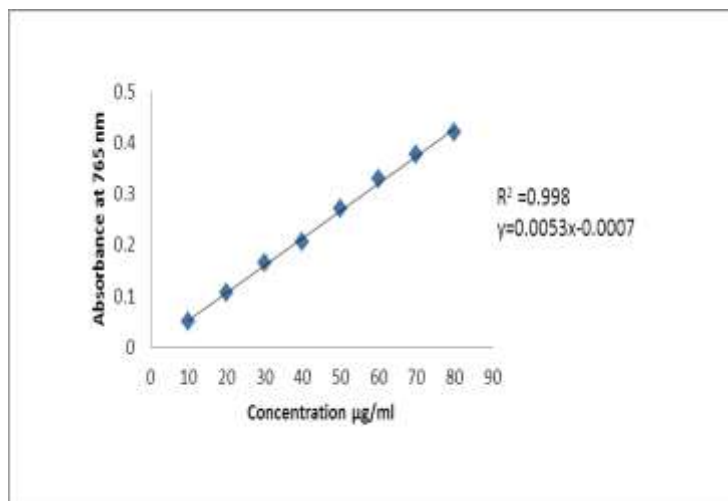


Figure No. 2: Calibration curve of Gallic acid

Calculation of total phenolic content of the extracts was done by using calibration curve equation: $y = 0.0053x - 0.0007$, $R^2 = 0.998$ obtain by plotting calibration curve of standard Gallic acid where y was the absorbance and x was the concentration. The following bar diagram shows the total phenolic content of different extracts as mg Gallic acid equivalent (GAE) per gram.

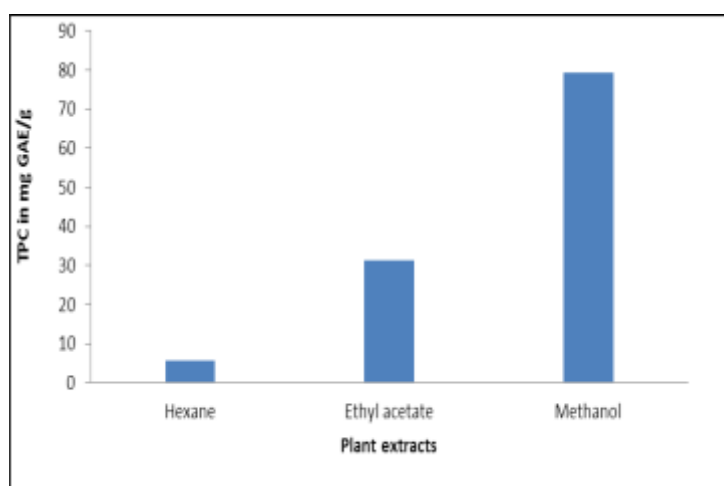


Figure No.3: Total phenolic content of different extract of plant (mgGAE/g)

Total Flavonoid Content determination

Calibration curve of standard Quercetin was obtained by Microsoft Excel 2007 where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

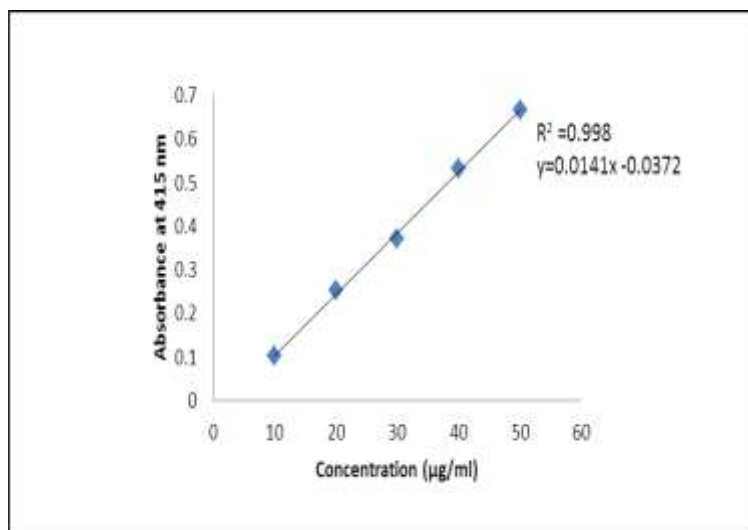


Figure No.4: Calibration curve of standard Quercetin

Calculation of total polyphenolic content of the extracts was done by using calibration curve equation: $y = 0.0141x - 0.0372$, $R^2 = 0.998$ obtain by plotting calibration curve of standard Quercetin where y was the absorbance and x was the concentration. The following bar diagram shows the total flavonoid content of different extract as mg Quercetin equivalent (QE) per gram.

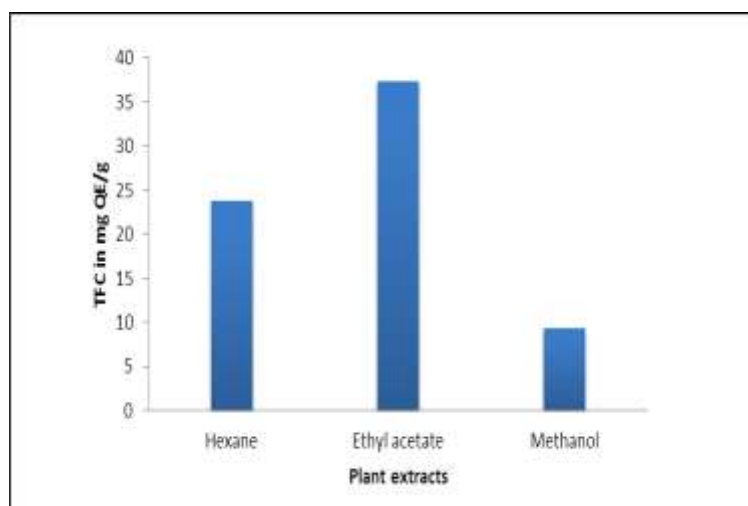


Figure No.5: Total flavonoid content of different extracts

Antioxidant Activity

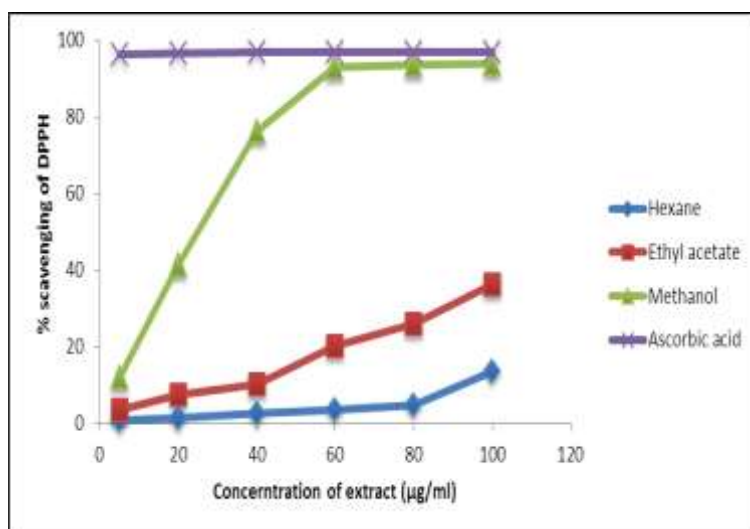


Figure No.6: DPPH scavenging of different extracts of plant

Antimicrobial Activity

The following table shows the result of preliminary antimicrobial activity of plant extracts. The results showed extract were active against *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhi* but not significant in *Escherichia coli*. Methanol extract showed comparable zone of inhibition with positive control against *Bacillus cereus* and *Staphylococcus aureus* then it was subjected for determination of minimum inhibitory concentration and minimum bactericidal concentration. The result showed that MIC and MBC value for *Bacillus cereus* was 6.25mg/ml and *Staphylococcus aureus* was 25mg/ml.

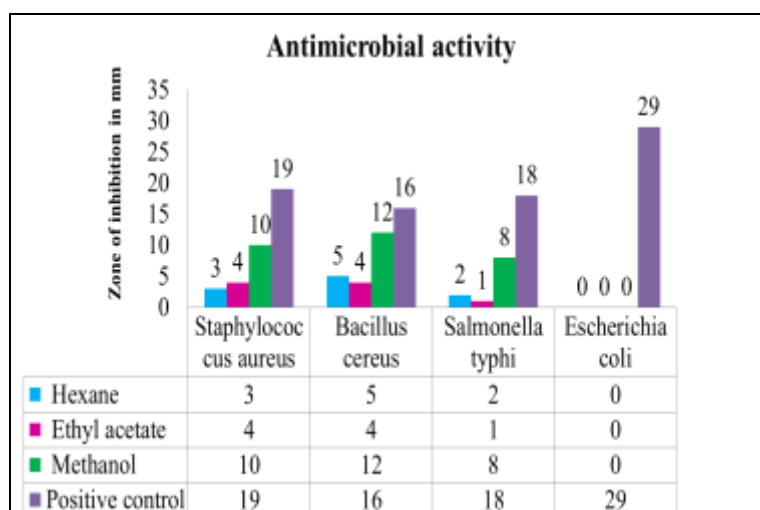


Figure No. 7: Antimicrobial activity

Zone of inhibition in mm

Control = 50 μ g/ml ofloxacin

Concentration of extract= 100mg/ml

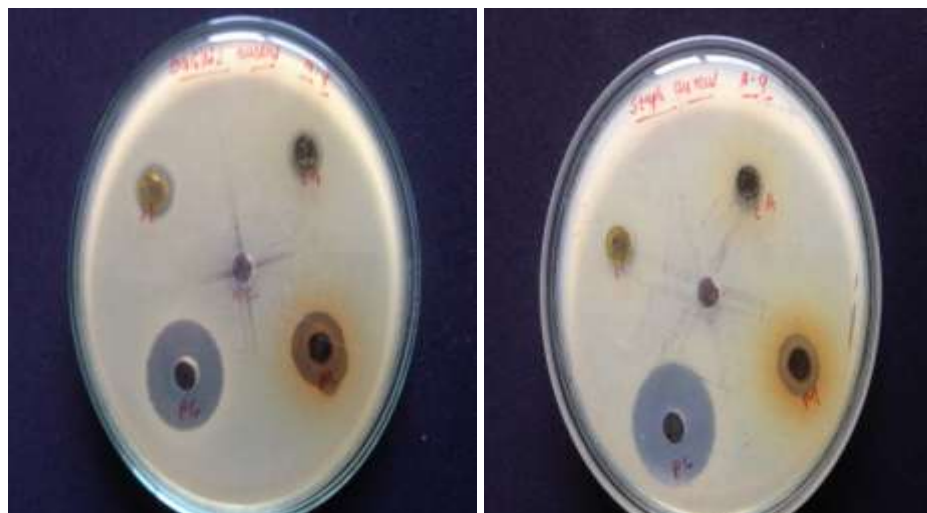


Figure No.8: Antimicrobial activity, treated with *Aleuritopteris anceps* on different organisms

Hypoglycaemic activity analysis with glucose loading rats

Glucose administration 3gm/kg glucose led to elevation of blood glucose level. After treatment of 200mg/kg, 400mg/kg methanol extract of *Aleuritopteris anceps* and 0.43mg/kg Glimiperide led to a dose dependent fall in blood glucose levels.

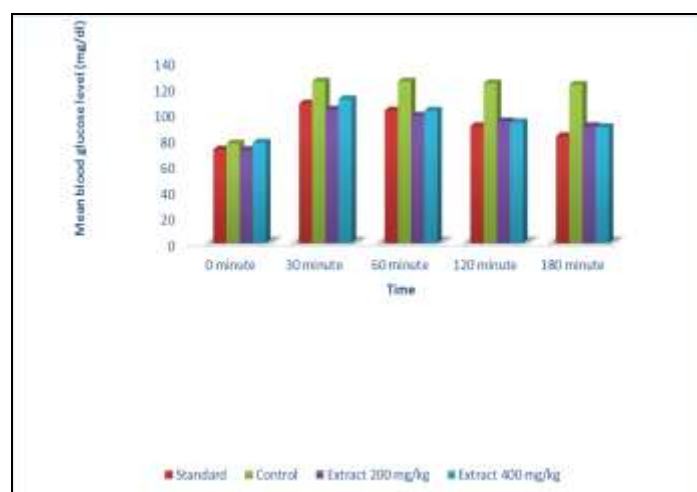


Figure No 9: Hypoglycaemic activity of different concentration of methanolic extract and Glimiperide

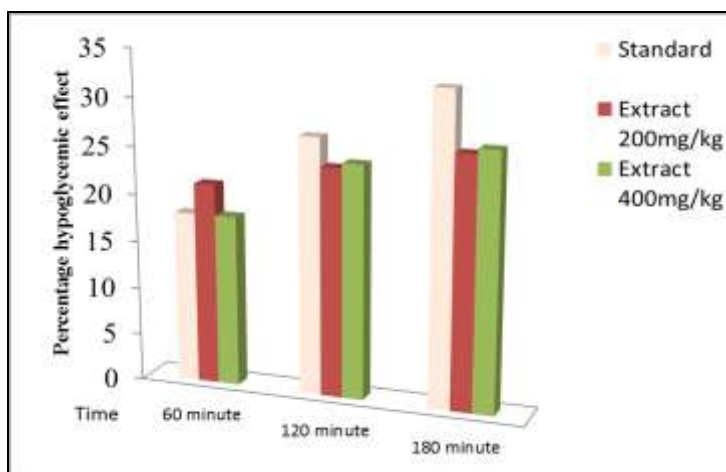


Figure No.10: Percentage hypoglycemic effect



Figure No 11: Hypoglycemic effect, treated with *Aleurites anceps* on glucose loaded Rats

DISCUSSION

The present study deals with phytochemical screening and biological evaluation of *Aleurites anceps*. The whole plant material was successively extracted with hexane, ethyl acetate and methanol (in increasing nonpolar to polar).

The preliminary phytochemical screening of extracts of plant was found to exhibit positive alkaloid, saponins, glycosides, terpenoids, tannins, carbohydrate, flavonoids and phenols. The phytochemical test results obtained methanol extract exhibit positive alkaloid saponins, glycosides, terpenoids, tannins, carbohydrate, flavonoids and phenols similarly ethyl acetate extract showed positive test for alkaloid, saponins, glycosides, terpenoids, tannins, carbohydrate, flavonoids and phenols. The study showed ethyl acetate and methanol extract exhibit positive for maximum test compounds except proteins whereas hexane extract showed all the phytoconstituents studied except saponins and proteins. The differences

indicate the insolubility of certain group of compounds in certain solvents. Thus phytochemical screening helped in identifying the main constituent present in the extract.

A study on aqueous ethanolic extract of plant conducted in India was showed positive for carbohydrate and flavonoid.^[30] Similar study conducted in India was also showed presence of flavonoid glycoside of the plant.^[31] The previous research work was in chromatographic fractionated compound but my study was in crude extract however both results were similar, it may be similarities in geographical distribution.

Total Phenolic Content (TPC) and Total Flavonoid Content of the extract were expressed as milligram of Galic Acid Equivalent per gram of extract i.e. mgGAE/g of extract and milligram of Quercetin Equivalent per gram of extract i.e. mgQE/g of extract respectively.

The amount of total phenolic and flavonoid content of *Aleuritopteris anceps*, was measured by Folin-Ciocateau and colorimetric assay, varied in different extracts and ranged from 5.60 in hexane, 31.26 in ethyl acetate and 79.19 mgGAE/g in methanol. The study showed higher TPC value was found in methanol, ethyl acetate and hexane respectively.

The Total Flavonoid Content was found 23.77 in hexane, 37.22 in ethyl acetate and 9.38 mgQE/g in methanol. The result showed higher amount in ethyl acetate, hexane and methanol respectively. Previous research effort on TPC and TFC determination of plant *Aleuritopteris anceps* was not found.

A study reported that phenolic compound have an antioxidant activity due to ability to scavenging of free radicals such as Reactive Oxygen Species (ROS). Amount of phenolic compounds are responsible for the antioxidant activities.^[32]

As per previous research study, a research conducted in India by Mishra & Verma 2009, chromatographic isolated and identified two flavonoid glycoside showed antioxidant activity. Similar study conducted by Chowdhary et al., 2010 showed antioxidant activity. Result obtained from this study was similar to previous literature; however this extract was crude extract, this may be due to same geographical distribution.

The antimicrobial screening of different extracts of plant showed activity against the gram positive and Gram negative bacteria. The result showed activity on *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhi* but none of extracts showed activity at concentration

100mg/ml against *Escherichia coli*. Methanol extract showed comparable zone of inhibition with positive control against *Bacillus cereus* and *Staphylococcus aureus* then it was subjected for determination of minimum inhibitory concentration and minimum bactericidal concentration. The result showed that MIC value for *Bacillus cereus* was 6.25mg/ml and *Staphylococcus aureus* was 25mg/ml.

Literature has been shown that terpenoids and phenolic compound shows most of the antimicrobial activity. Thus the activity of plant extracts against bacteria may be due to phenolic compound or terpenoid compound or combination of both.^[33] Study showed that different extracts of *Aleuritopteris anceps* contains phenols, terpenoids and flavonoid compounds and also possesses antibacterial activity it may be due to phenolic, terpenoids or flavonoids. The phenolic toxicity to microorganisms may be due to enzyme inhibition. Flavonoid compounds exert their activity due to their ability to complex with extracellular soluble proteins, bacterial cell wall and microbial membrane disruption by lipophilic compounds. The exact mechanism of action of terpenoids is not clear but it may exert its activity by membrane disruption.^[34] But nothing can be said unless exact compound responsible for such activity is isolated.

In antihyperglycemic activity tests, the methanolic extract was observed to produce a dose dependent lowering of glucose concentration in blood higher in 180 minute after glucose – loaded rats. The percent reductions in blood glucose concentrations when the extract was administered at dose 200 and 400 mg per kg body weight of rats were respectively, 26.23% and 26.79% as compared to control rats, which did not receive any extract or antihyperglycemic drugs. A standard antihyperglycemic drug, glimepiride, when administered at a dose 0.43mg/kg body weight to rats, was observed to lower blood sugar by 32.52%, when compared to control rats (i.e. rats administered distilled water only). Thus the antihyperglycemic activity of the extract can be considered to be comparable to glimepiride, the glimepiride reduces the plasma glucose level by stimulating the insulin production of pancreatic β cell through inhibition of K^+ channels and decreasing the sensitivity of peripheral tissue to insulin, but nothing can be said the exact mechanism of hypoglycaemic effect however there may be possibilities of similar action as glimepiride or reduction of glycogenolysis or inhibit glucose absorption in gut or combination of both. Thus, it may be added that observed results support the folk medicinal use of the plant for antidiabetic effect.

This study exposed as a preliminary analysis of the various extracts of the plant *Aleuritopteris anceps* has significant phytochemical screening and biological study of the plant. From this study, it is not possible to point the exact phytoconstituent (s) responsible for different biological activity, further phytochemical and biological study are needed for the identification of main phytoconstituent (s) are responsible for these activities.

CONCLUSION

This study exposed as a preliminary analysis of the various extracts of the plant *Aleuritopteris anceps* has significant phytochemical screening and biological study of the plant.

From this study, it was found that the plant possesses different phytochemical constituents like alkaloids, saponins, glycosides, terpenoids, flavonoids, tannins, carbohydrates and phenols. The plant has significant antioxidant activity with high amount of total phenolic and flavonoid content. This could be a good source of natural antioxidant. Antimicrobial activity against gram positive and gram negative bacteria was significant in *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhi* but not significant in *Escherichia coli*. The biological study conducted in animal revealed significant hypoglycemic activity.

It can be concluded that the plant possesses certain medicinal values which may generate the lead molecules for development of newer drugs candidate. Considering these promising biological activities shown by the plant extracts, further phytochemical and biological studies to identify the principle constituents responsible for these activities are highly recommended for better evaluation of the bioactivity potential of the plant, so that its phytochemicals can be utilized as an alternative medicine in future.

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REFERENCE

1. Baral, S. R., & Kurmi, P. P. (2006). Compendium of medicinal plants in Nepal.
2. Sen, P. (1993). Therapeutic potentials of Tulsi: from experience to facts. *Drugs News & Views*, 1(2): 15-21.
3. Acharya, E., & Pokhrel, B. (2006). Ethno-medicinal plants used by Bantar of Bhaudaha, Morang, Nepal. *Our Nature*, 2006; 4(1): 96-103. *Our Nature*, 4(1): 96-103.
4. Joshi, B., Lekhak, S., & Sharma, A. (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu university journal of science, engineering and technology*, 5(1): 143-150.
5. Singh, A. G., & Hamal, J. P. (2013). Traditional phytotherapy of some medicinal plants used by Tharu and Magar communities of Western Nepal, against dermatological disorders. *Scientific World*, 11(11): 81-89.
6. Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. *J. Clin. Pathol.*, 54: 176-186.
7. Djeridane, A., Yousfi, M., & Nadjemi, B. (2006, August). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97(4): 654-660.
8. Di Carlo, G., Mascolo, N., Izzo, A. A., & Capasso, F. (1999, June). Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sciences*, 65(4): 337- 353.
9. Savithramma, N., Rao, M. L., & Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*, 8(3): 579-584.
10. De, S., Dey, Y. N., & Ghosh, A. K. (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). *Int J Pharm Biol Res*, 1: 150-157.
11. Adedapo, A., Jimoh, F., & Afolayan, A. (2011). Comparision of the nutritive value and biological activity of the acetone, methanol and water extracts of the leaves of *Bindens pilosa* and *Chemopodium album*. 68(1): 83-92.
12. Piaxao, N., Perestrelo, R., Marques, J. C., & Camara, J. S. (2007). Relationship between antioxidant capacity and total phenolic content of red rose and white wines. *Food Chemistry*, 105: 204-214.
13. Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Techno*, 26(2): 211-219.

14. Sahu, R. K., Kar, M., & Routray, R. (2013). DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribals of Odisha, India. *J. Med. Plants*, 1(4): 21-27.
15. Chew, A. L., Jessica, J. A., & Sasidharan, S. (2012). Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pacific journal of tropical biomedicine*, 2(3): 176-180.
16. Srivastava, J., Lambert, J., & Vietmeyer, N. (1996). Medicinal plants: An expanding role in development. *World Bank Technical Paper*, 320.
17. Uniyal, S. K., Singh, K. N., Jamuwal, P., & Lal, B. (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. *J. Ethnobiol. Ethnomed.*, 2: 1-14.
18. Kumarasamy, K. k., Taleman, M. A., Walsh, T. R., Bangari, J., Butt, F., Balkrishna, R., Neil, w. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan and the UK: a molecular, biological and epidemiological study. *The Lancet Infectious Diseases*, 10(9): 597-602.
19. Khanam, Z., Wen, S. W., & Haq Bhat, I. U. (2015). Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). *Journal of King Saud University- Science*, 27: 23-30.
20. Subedi, A., Amatya, M. P., Shrestha, T. M., Mishra, S. K., & Pokhrel, B. M. (2012). Antioxidant and antibacterial activity of methanolic extract of *Machilus odoratissima*. *Kathmandu University Journal of Science, Engineering and Technology*, 8(1): 73-80.
21. Joshi, B., Sah, G. P., Basnet, B. B., Bhatt, M. R., Sharma, D., Subedi, K., & Malla, R. (2011). Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *J Microbiol Antimicrob.* 3(1): 1-7.
22. Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract*, 87(1): 4-14.
23. Petal, M., & Rybczynski, P. T. (2003). Treatment of non-insulin dependent diabetes mellitus. *Expert Opin Invest Drugs*, 12(4): 623-633.
24. Chandramohan, G., Ignacimuthu, S., & Pugalendi, K. V. (2008). A novel compound from *Casearia esculenta* (Roxb.) root and its effect on carbohydrate metabolism in streptozotocin-diabetic rats. *Eur J Pharmacol*, 590(1-3): 437-443.
25. Bailey, C. J., & Day, C. (1989). Traditional plant medicines as treatment for diabetes. *Diabetes Care*, 12: 553-564.

26. Latha, M., & Pari, L. (2004). Effect of an aqueous extract of *Scoparia dulcis* on blood glucose, plasma insulin and some polyol pathway enzymes in experimental rat diabetes. *Brazilian journal of medical and biological research*, 37(4): 577-586.
27. Kumar, R., Pate, D. K., Prasad, S. K., Sairam, K., & Hemalatha, S. (2011). Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii* thwaites on streptozotocin-nicotinamide induced type 2 diabetic rats. *Asian Pacific Journal of Topical Medicine*, 904-909.
28. Ahmed, F., Rahman, S., Ahmed, N., Hossain, M., Biswas, A., Sarkar, S., & Khatun, A. (2011). Evaluation of *Neolamarckia cadamba* (Roxb.) Bosser leaf extract on glucose tolerance in glucose-induced hyperglycemic mice. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1).
29. Faisal, M., Hossain, A. I., Rahman, S., Jahan, R., & Rahmatullah, M. (2014). A preliminary report on oral glucose tolerance and antinociceptive activity tests conducted with methanol extract of *Xanthosoma violaceum* aerial parts. *BMC complementary and alternative medicine*, 14(1): 335.
30. Mishra, R., & Verma, D. L. (2009). Flavonol glycosides of *Cheilanthes anceps* Roxb. *journal of American science*, 5(4): 183-188.
31. Chowdhary, S., Verma, D. L., Pande, R., & Kumar, H. (2010). Antioxidative properties of flavonoids from *Cheilanthes anceps* Swartz. *Journal of American Science*, 6(5): 203-207.
32. Al-Mustafa, A. H., & Al-Thunibat, O. Y. (2008). Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes. *Pak J Biol Sci*, 11(3): 351-358.
33. Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, 88(2): 308-316.
34. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, 12(4): 564-582.