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Phytochemical and antimicrobial screening of indigenous species that have potential for revegetation of landslides in Atok, Benguet, Philippines

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Atok, Benguet in Northern Philippines is highly susceptible to landslides. To mitigate the effects of landslides, revegetation, was recently introduced as a means of soil stabilization. The selection of revegetation species is crucial; they should be indigenous to the area, reproduce rapidly, and suitable for bioengineering. The choice of revegetation species is sometimes dependent on the local community. Hence, to encourage the community to use these plants, the researchers conducted this study to establish additional value, specifically for their phytochemical content and antimicrobial potential.The selected plants are *Coffea arabica*, *Brugmansia suaveolens, Camellia sinensis, Sarcandra glabra and Coriaria intermedia,* which are found in the adjacent areas of landslide scars. Results showed that *Camellia sinensis* have the most number of secondary metabolites. Further, the plant extracts were tested against five bacteria namely *Salmonella typhimurium*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using disc diffusion method. Leaf extract from *C. intermedia* was active against all the strains and is comparable to the results of the four antibiotics (Chloramphenicol, Vancomycin, Kanamycin and Streptomycin) used. The antibacterial potential and the presence of bioactive components of the different plants can increase the value of the plants both for economic and medicinal purposes.

Keywords: Antimicrobial activity, Atok, Disc diffusion, Indigenous, Phytochemical screening

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The municipality of Atok, from where plants in this study were collected, is one of thirteen municipalities of Benguet province. It is located at 16° 34' 56" North, 120° 41' 55" East of the Philippines. The people of Atok belong to different ethnic groups like *Ibalois, Kankana-eys* and *Kalangoyas* whose major source of income is farming[1]. According to the Geological Database Information, Atok, Benguet has high susceptibility to landslide which can be due to the frequency of heavy rains and the mountainous topography of the area[2].

The municipality, despite its susceptibility to landslide, hosts several flora species. The ethnic tribes have made use of this abundance by using them in various ways, some as herbal remedies to cure some ailments, some for fuel, and others[3]. With the presence of many landslide scars in the municipality, some of these species have the potential to be used in mitigating landslides through revegetation. In order to promote these plants for such purpose, additional uses as source of herbal medicine can be exploredto increase their value or importance. In this study, screening of these plants for their phytochemical content and antibacterial activity were focused.

Studies nowadays are seeking to explain the relationship of the plant's properties to its application as herbal medicine. The two most effective methods commonly employed to determine the plant's potential for medicinal purposes are phytochemical screening and test for antimicrobial or antibacterial activity. Phytochemical screening is being done to determine the primary and secondary metabolites present in the plants to be studied. Primary metabolites are the compounds involved in the metabolic pathways, which are common to all living organisms[4]. On the other hand, secondary metabolites are compounds that function as defense and signal compound that are necessary for the plant's survival

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and reproductive ability[5]. There were several studies performed in different countries which relate the presence of the secondary metabolites and antimicrobial activities to the local uses of the plants to cure some infections and illnesses [6-8]. In the Philippines, specifically in the Cordillera, similar but few studies were done to determine the scientific basis of the plants used for medicinal purposes by some ethnic tribes. In Sablan, Benguet, phytochemical screening and antibacterial tests were done on some plants[9,10].

In this study, the following objectives were set: (1) to perform phytochemical screening for the determination of the plants' secondary metabolites and (2) to study the antimicrobial activities of indigenous plant species found and commonly used in the locality of Atok, Benguet which also have potential as revegetation species. These plant species can be more beneficial to the community if found to have medicinal value. In addition, since these plants were found thriving in the locality, which is one of the requirements for the selection of plants for revegetation purposes, these species can be used in other studies to serve multiple purposes.

Methodology

Collection and preparation of plant materials

A landslide scar in Poblacion, Atok was surveyed and plants with potential as revegetation species were determined. The selected plant species were *Coffea Arabica* L., *Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl, *Camellia sinensis* (L.) Kuntze, *Sarcandra glabra* (Thunb.) Nakai syn. *Ardisia japonica* (Thunb.) Blume, *Coriaria intermedia* Matsum. syn. *Coriaria japonica* subsp. *intermedia* (Matsum.) T.C.Huang. These species were found in the adjacent areas of the landslide and some were used by the community as fence along the slope to help prevent soil movement.

Samples of the different parts, such as leaves, flowers, roots and stem, for each plant were collected. The plant samples were cleaned, diminuted and air dried for one week. After drying, 100 gm of each sample was then macerated at ambient temperature using 100 ml of 95% methanol for 24 hrs. The mixtures were then filtered and the filtrates were concentrated by reducing the volume to about 20% of the original volume over water bath at 40°C. The concentrated crude extracts were used for phytochemical screening and antimicrobial study.

Phytochemical screening

Phytochemical screening for secondary metabolites was performed following standard procedures[11]. The tests for flavonoids are Bate-Smith and Metcalf and Wilstatter methods. For saponins, froth test was done. To identify the presence of tannins and polyphenols, ferric chloride test was followed. Guignard test was employed to qualitatively determine the cyanogenic glycoside. Dragendorff's test, Keller-Kiliani test and modified Borntrager's test were done to test for the presence of alkaloids, steroids and anthraquinones, respectively.

Antimicrobial study

Test organisms

The test organisms used in this study, *Salmonella typhimurium*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa,* were obtained from Natural Science Research Institute of University of the Philippines Diliman. These organisms are common human pathogens that can cause several diseases from simple boils to food intoxication and toxic shock syndrome (*S. aureus*), intestinal and urinary tract infections (*E. coli, S. typhimurium, P. aeruginosa*) and food poisoning (*B. cereus*)[12].

Preparation of culture media and microbial inocula

The base agar was prepared following the same instruction indicated in the label. Preparation of the inoculum was done by taking a loopful of test organism from the stock and suspended in sterile saline solution to achieve the same turbidity as that of 0.5 McFarland standard. Modification in the preparation of the Mueller-Hinton plate was done. A top layer composed of the prepared inoculum and soft agar solution (prepared by using half the amount of original agar quantity and same amount of water) was poured over the base agar and was allowed to solidify.

Antibacterial assay

The standard protocol employed for antibacterial study is the Kirby-Bauer disk diffusion test. This test was done to determine the resistance or the susceptibility of test microorganisms to the compounds present in the plant extracts. The disks with 5µL test extracts were then placed on the petri plates with the inoculum. Also, standard antibiotic discs namely Vancomycin (30µg), Kanamycin (30µg), Streptomycin (10µg) and Chloramphenicol

(30µg) were tested against the same pathogens and methanol was used as the negative control. The plates were incubated at 37°C for 24 hrs and diameters of zones of inhibition were measured in millimeters with the aid of a ruler. The antimicrobial activity test was done in triplicates. The absence of growth around the disks is an indirect measure of the ability of the compound to inhibit the microorganisms' growth[13].

Results and discussion

Phytochemical screening

Phytochemical screening results presented in Table 1 showed that the plants contain various secondary metabolites including flavonoids, saponins, tannins, alkaloids, steroids and glycosides. In particular, the presence of flavonoids was noted in all samples and was very prominent in the methanolic extracts of *Camellia sinensis* and *Coffea arabica* leaves. Flavonoids have been known to exhibit many biological activities such as antimicrobial, photo receptors, feeding repellants but most studies focused on the flavonoids' ability as antioxidant[14]. Tannins were also observed to be present in all the plant extracts in this study. This group of metabolites has shown antimicrobial, antidiarrheal and anthelmintic properties[15]. Interestingly, anthraquinone was found to be negative for all the plant extracts tested. The reason can be that the solvent used, which is 95% methanol, was not effective on extracting anthraquinone compounds. This is supported by a study done in extracting anthraquinone *from Senna alata,* water concentration at 15% enhanced the anthraquinone extraction by 1.47 times compared to the extraction without water[16].

The presence of secondary metabolites, which are not necessarily produced under different environmental conditions, is an expression of the individuality of species. These secondary metabolites provide the most pharmacologically active natural products since they are present in plants in high structural diversity - which is now considered due to the adaptation of the plants to its environment[4]. Each class of metabolites has thousands of different compounds that give different phytochemical properties of the plant species[5]. Due to these reasons, plant species found in one place may have different compounds or secondary metabolites compared with that found in another place or area. In this study, only the general classifications of the secondary metabolites were determined.

Among the collected indigenous plant materials, *Camellia sinensis*, locally known as *itsaa*, has the most number of secondary metabolites present such as flavonoids, alkaloids, cyanogenic glycosides, saponins and tannins, except anthraquinones. The result of this study is similar to that of done by Farrukh[17]. The presence of several metabolites in *Camellia sinensis*, commonly used as green and black tea, might be responsible to its several health benefits including increasing mental awareness, treatment of genital warts and preventing some kinds of cancer[18].

From the results obtained for *C. arabica*, the secondary metabolite alkaloid – the group where the active component caffeine belongs, is present in the leaf extract but not in the fruit extract. The result is similar to the phytochemical screening of *Coffea arabica* leaves in Sablan, Benguet done by Balangcod *et al.*[9]. The probable reason can be that the amount of caffeine varies in different species and within species.

Table 1—Results of Phytochemical screening for secondary metabolites

Extract	Flavonoids	Saponins	Tannins	Alkaloids	Steroids	Anthraquinones	Cyanogenic glycosides
Camellia sinensis leaves	(+++)	(+)	(+)	(++)	(+)	(-)	(+)
Coffea arabica leaves	(+++)	Slightly (+)	(+)	(+)	(+)	(-)	(+)
Coffea arabica fruit	(+)	(-)	(+)	(-)	(-)	(-)	(-)
Brugmansia sp. stem	(+)	(-)	(+)	(-)	(-)	(-)	(-)
Brugmansia sp. leaf	(+)	Slightly (+)	(+)	(++)	(-)	(-)	(-)
Brugmansia sp. flower	(+)	(-)	(+)	(+)	(+)	(-)	(-)
Coriaria intermedia leaves	(+)	(-)	(+)	(+)	(-)	(-)	(+)
Coriaria intermedia flower	(+)	(-)	(+)	(+)	(-)	(-)	(-)
Sarcandra glabra leaves	(+)	Slightly (+)	(++)	(-)	(-)	(-)	(+)

Table 2—Average Zone of Inhibition (in mm) of the different extracts against test microorganisms

Extract	*Salmonella typhimurium*	*Bacillus cereus*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Camellia sinensis leaves	10	9	12	14	14
Coffea arabica leaves	0	0	0	0	0
Coffea arabica fruit	0	0	13	0	0
Brugmansia sp. stem	0	0	0	11	0
Brugmansia sp. leaf	0	0	0	0	0
Brugmansia sp. flower	0	0	0	0	0
Coriaria intermedia leaves	13	11	11	14	12
Coriaria intermedia flower	10	9	9	11	12
Sarcandra glabra leaves	0	0	0	0	0

Table 3—Zone of Inhibition and Its interpretation

Zone of Inhibition, in mm	Interpretation
<10	Inactive
10 – 13	Partially Active
14 – 19	Active
> 19	Very Active

Likewise, the caffeine contents differ in different parts of the plants and at different growth stages[19].

Antimicrobial study

The results of antimicrobial activity of the crude medicinal plant extracts are presented in Table 2. Those that were considered as inactive against all test microorganisms were the extracts of *Coffea arabica* leaves, *Brugmansia suaveolens* leaf and flower, and *Sarcandra glabra* (*Gipas*) leaves. The most prominent result was the extract of *Coriaria intermedia* leaves. It was considered partially active against *S. typhimurium, B. cereus, E. coli, and P. aeruginosa;* and active against *S. aureus*. The extract of *itsaa* leaves is second to that of *C. intermedia* leaves. *Camellia sinensis* extract was inactive against *B. cereus*; partially active against *S. typhimurium* and *E. coli* and was active against two pathogens namely *S. aureus* and *P. aeruginosa.* The *C. arabica* fruit extract, which is commonly converted to coffee granules or powder, was partially active against *E. coli*. The interpretation of the antibacterial results was based on the standard set by Quinto[20], which is shown in Table 3. Further, in order to establish the extracts' antimicrobial effectivity, the results were compared against known antibiotics namely Vancomycin, Kanamycin, Streptomycin and Chlorampenicol. Methanol was used as the negative control. Table 4 below shows the zone of inhibitions for the positive and negative controls. The results of the positive controls ranged from active to very active against the five pathogens tested. Methanol, which is the negative control, showed no activity against all the pathogens used.

Fig. 1 showed the zone of inhibitions exhibited by the extracts of *Brugmansia suaveolens* leaves (9B), *Brugmansia suaveolens* flower (10B), *Coriaria intermedia* leaves (11B) and flowers (12B). The photo on the right was that of the controls used namely Vancomycin (C1), Kanamycin (C2), Streptomycin (C3), Chloramphenicol (C4) and methanol (C5). Specifically, among the plants tested, the methanolic extract of *C. intermedia* has the most activity against the pathogens relative to *C. sinensis*. Its antimicrobial activity against *S. aureus* is comparable to those of the four antibiotics used. The zone of inhibition on *E. coli* of *C. intermedia* was very near to that of Vancomycin, having 11 and 14 mm clearing zones, respectively. In comparison, the species found in Atok, Benguet has different antimicrobial activity from those found in the Visayas and Mindanao regions. Based on the study done by Tesoro[21], the first fraction out of four fractions from gradient partitioning of methanol extract of *Coriaria intermedia* showed activity against *P. aeruginosa* and *B. subtilis* but inactive against *S. aureus* and *E. coli*.

Generally, the activities of the plant extracts against all the test organisms can be attributed to its secondary metabolite contents. In this study, *C. intermedia* contain flavonoids, tannins and alkaloids. Flavonoids are phenolic group of metabolites that are known to be synthesized by plants in response to microbial infection and so are found to be effective antimicrobial agent against different microoganisms.

Table 4—Average Diameter of Zone of Inhibition (in mm) of Antibiotics and Methanol

Antibiotic	*Streptococcus typhimurium*	*Bacillus cereus*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Vancomycin	26	21	14	17	26
Kanamycin	28	21	21	19	28
Streptomycin	34	20	20	18	20
Chloramphenicol	23	21	18	16	32
Methanol	0	0	0	0	0

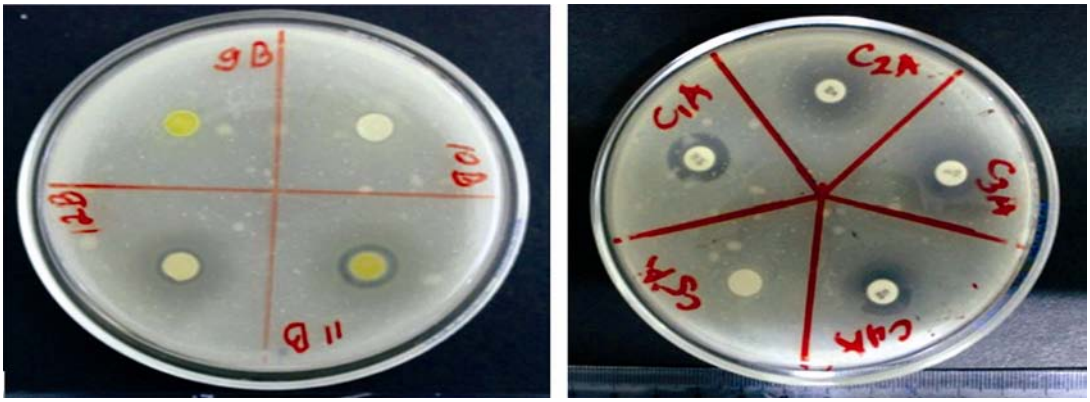

Fig. 1—A sample of zone of inhibitions of the plant test extract (left) and the antibiotic standards (right) against *E. coli*

This is due to their ability to complex with bacterial cell walls. Tannins, also a phenolic compound, have the ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins while alkaloids can inhibit bacterial growth by intercalating with DNA[22].

Camellia sinensis, the plant species found to have the most number of secondary metabolites compared to other species in this study, was active against two pathogens - *S. aureus and P. aeruginosa*. This result was comparable to that of Steptomycin in view of the activity against *P. aeruginosa*. For the activity against *S. aureus*, the result of *Camellia sp*. extract was comparable to all antibiotics used like the *Coriaria* sp. extract. Moreover, the results of Vancomycin and *C. sinensis'* activity are very near to each other. *C. sinensis* leaves were found to contain saponins and steroids in addition to the metabolites found in *C. intermedia*. Saponins were found to be capable of damaging the membrane of cellular materials that lead to cell's death as cited by Al-Bayati[23]. The results of this study is in agreement with that of another study done by Tariq A *et al.*[24], which showed that *Camellia sinensis* extract has alkaloids, flavonoids, steroids, and tannins and showed activity against *B. subtilis* and *Enterococcus* sp.

Although the extracts from *Brugmansia suaveolens* and *Sarcandra glabra* from Atok, Benguet showed no antimicrobial activity in this study despite the presence of secondary metabolites, there have been other reports that showed antimicrobial properties. In particular, ethanol extract of *Sarcandra glabra* was found to inhibit the growth of *A. baumannii, P. aeruginosa* and *S. aureus*[25]. In a study done by Schmidt *et al.*[26], *Brugmansia suaveolens* showed activity against *B. subtilis* which is comparable to the vancomycin standard used. Also, *Coffea* sp. which contains flavonoids and tannins, as previously mentioned, has not inhibited the growth of the test microorganisms used. This is in contrast to the antimicrobial activity of the *Coffea* sp. from Sablan, Benguet, which was partially active against *E. coli, P. aeruginosa and S. typhimurium*; and active against *S. aureus*[9].

Based on the above comparison of the results with other studies, the presence of secondary metabolites and antibacterial activities of the same plant species from different locations can vary. The presence of certain metabolites reflects adaptation and reaction of the plants for their survival, so if the plants are exposed to different environments and stresses, they will develop different reactions and metabolic compounds. Also, the existence of the same secondary metabolites does not necessarily entail the same antimicrobial activity, as in the case of the

C. arabica collected from Atok and Sablan, Benguet. Consequently, the same species found in separate locations can have the same groups of metabolites but can differ in antimicrobial activities. From this, it can be inferred that different specific compounds, which may belong in the same group and are found on the same species, can have different antibacterial responses. This is because the metabolites can exhibit thousands of different chemical structures. Thus, it is important to do the basic phytochemical screening and antimicrobial activity test even if several studies were reported regarding the species to be tested.

Moreover, even if the test extracts of the plant species from Atok, Benguet do not have the same effectivity as those of standard antibiotics, antibacterial potential should not be disregarded. The amount of extracted metabolites or active components might not be enough to inhibit the pathogens. Also, there could be some other pathogens, which were not used in this study, that are susceptible to the extracts.

Since the presence of secondary metabolites and antibacterial activities have been established in this study, the plant species tested can be tapped for dual purposes, for medicinal cures and for revegetation. This will be beneficial to the local community by having an affordable and accessible source of medicine and a less expensive means of soil stabilization.

Conclusion

Based on the results obtained, all the indigenous plants tested, which have revegetation potential, contain flavonoids and tannins that have reported antimicrobial activities. The presence of these compounds and the antibacterial activity they exhibited provide a basis for their potential uses as herbal medicines, thus increasing their importance as revegetation species as well. Therefore, these plant species can serve dual purposes to the community - as potential medicinal sources and revegetation species for landslide prevention. It is recommended that other plants which have dual purposes can be explored.

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