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Indigenous Knowledge Meets Science: A Saponin Phytochemical Screening of Folk Medicines Used for Treating Cough and Colds in *Cagraray* Island, Philippines

Michael B. Bibon

Cawayan National High School, Department of Education-Albay Divison, Legazpi City, Albay, Philippines

Author's Mail Id: michael.bibon@deped.gov.ph, Tel.: +63975-551-3891

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Abstract—Shortcomings on access to quality health care professionals and facilities caused the locals of *Cagraray* island to rely on folk medicines for treating cough and colds. These illnesses were often treated by the ingestion of antibacterial medicines, expectorants, and antitussives. Saponin, an antibacterial chemical and expectorant, exists in some plant extracts in a form of phytochemical. This study screened the saponin content of folk medicines in *Cagraray* island used for treating cough and colds. Field immersion through survey and interview was conducted to 8 folk healers considered as key informants of the study. Saponin Foam Test was administered for 3 trials to the prescribed 9 folk medicinal herbs to test the levels of saponin and subjected for quantitative analysis. The screening test revealed the presence of the phytochemical saponin in varying quantities across tested medicinal herbs. This was supported by ANOVA indicating significant difference in the levels of saponin from different folk medicinal herbs tested [*F* (8, 18)=122.47, *p*<0.05]. The result concluded a strong link between science and indigenous knowledge by proving the potentiality of the folk medicines to treat cough and colds. Nonetheless, the research recommends to further study the phytochemical contents of the identified medicinal plants, with or without saponin, to testify the truthfulness of their healing effect and toxicity levels.

Keywords—Cough and Colds, Cagraray Island, Indigenous Knowledge, Phytochemical Screening, Saponin Testing

I. INTRODUCTION

Cough is a common condition throughout the world characterized by the formation of sputum on the lining of windpipe, and contractions of the diaphragm. It is a condition prevalent and persistent for people with respiratory disorders [1]. Aside from prescribed antibiotics, its treatment is usually carried out by the ingestion of overthe-counter (OTC) commercialized medicines to alleviate the condition. These OTC drugs are categorized into suppressants, mucolytics, and expectorants [2]. Accounts from literature indicated that the major cause of the condition is the infection of upper respiratory tract caused by smoke, allergens [3], and microbial infestation [4]. Aside from viruses, cough and colds are often stimulated by bacteria [5], while the expulsion of mucus build-up needs a surfactant to act as expectorant [6].

Reference [7] noted that cough and colds were one of the most predominant illnesses experienced by the people of *Cagraray* island. Connected only by a bridge to the mainland *Bacacay*, the island's geographical location brought many shortcomings in the access to quality and modern health care system and facilities, in addition to the experienced poverty. Folk medicine has been the alternative for the majority of its people in treating cough and colds along with other illnesses that exist in the locale.

Instead of buying expensive synthetic medicines, its people rely to herbal prescriptions and prayer-based exercises as recommended by their native folk healers. In fact, the literature suggests that equivalent to 80% of world's population used herbal medicines for treating underlying conditions [8] where alternative medicines were perceived as cheap yet effective option for primary health care [9]. Nevertheless, usage and dependence of people in herbal medicine were well demonstrated based from the acceptance of people and their positive health properties [10, 11].

Saponin (Figure 1), an expectorant [12] and antibacterial agent [13], exists in many plant extracts in a form of phytochemical [14]. In fact, saponin is processed and contained in commercialized products as tablets and syrups used for treating common colds with cough [15]. Evaluating and harnessing the biological potential of this phytochemical can lead to the attestation of organic alternatives for treating cough and colds. Furthermore, this study implies that the use of herbs could possibly contribute in the herbs' healing ability by mere chances of either placebo or scientific evidences by studying their phytochemical constituents. Unlocking the true nature of folk medicine used for treating cough and colds could possibly testify for the truthfulness of the indigenous knowledge of using herbs proven to contain saponin. This

present study, however, discloses the fact that treatment for cough and colds is not solely attributed to saponin as there are countless list of phytochemicals that also act as expectorants and antibacterial agents contained in the extracts of identified herbs. The present study is significant by documenting the underlying scientific principles of indigenous knowledge on medicines which cover the existing gap on discovering effective and alternative organic drugs.



Figure 1. Molecular structure of a typical saponin from a soy bean [16].

The current study pursues the identification of folk medicinal herbs used for treating cough and colds in *Cagraray* island that contain Saponin. The study specifically aims to (1) identify the herbs used in *Cagraray* island as folk medicine for treating cough and colds, (2) determine the saponin content level of the herbs in three trials, and (3) determine the significance of difference in saponin levels across three trials, and for every herb. It is hypothesized that saponin content across all folk herbal medicines do vary.

II. RELATED WORKS

Saponin originated from a Latin word which means "soap" due to foamy formation when it is mixed with water [17]. Breakthrough in saponin study was made by reference [18] who noted that the chemical exhibits antifilarial, inhibition of diseases, biosynthetical, hemolytic, and antifungal activities.

Literature body had shown many uses of saponin. It was documented that the chemical extracted from *Melanthera elliptica* has antibacterial and antifungal efficacy against some of the known bacterial and fungal species as shown in the inhibition of their growth in an *in vivo* assay [19]. This was supported by another study showing the antibacterial activity of saponin extracted from *Hemidesmus indicus* against pathogenic organisms like *S. aureus, S. typhi, K. pneumonia, A. flavus, A. fumigatus,* and *A. niger* [20]. The damaging ability of saponin is

attributed to its capacity of binding to the cholesterol inside the cell making a complex saponin-cholesterol molecule that is capable of promoting lysis to cells [21].

In another study, the antitussive and expectorant properties of extracts from *Hedera helix* and *Rhizoma coptidis* were linked to saponin phytochemical as it was shown to be effective in cough inhibition among mice used in *in vivo* assay. [22]. This was further supported by a study documenting the literature on using and proving the efficacy of saponin-rich herbs for treating cough among children [23].

Recorded studies have also shown that saponin has anticancer properties by inducing apoptosis to chronic leukemia B cells [24]. The same findings were reported in a study of breast carcinoma cells and melanoma indicating an anticancer property of saponin extracted from *Allium chinense* [25].

In a similar study, reference [26] made a shortlist of herbs proven to exhibit antitussive and expectorant properties where saponin is one of the active phytochemicals contained in some of the documented folk medicinal herbs.

Literature accounts have also shown that saponin degrades when processed with the intervention of extreme heat [27].

The consolidate review of previously documented works showed that saponin from many plant species has a promising use in treating cough and colds, in addition to other recorded positive effects of the raw phytochemical in treating illnesses.

III. METHODOLOGY

Interview to Document Folk Medicinal Herbs

An interview to the folk healers of *Cagraray* island was conducted regarding the herbs they recommend for treating cough and colds. The study limits itself to the herbal prescriptions that were applied through ingestion whereas herbal poultices were disregarded. Eight folk healers were considered who were natives of *Cagraray* island, have been practicing folk healing for more than 15 years, and willing to participate in the study. The participation of the identified folk healers was recruited based from referral sampling technique through interviewing the locals of the island. Table 1 is the list herbal medicines unanimously recommended by the 8 folk healers for treating cough and colds.

 Table 1. Folk Medicinal Herbs. Herbs used for treating cough and colds.

| | and condor | | |
|-----------------------|---------------------|------------------------|--------------------------------|
| Herbs (Local name) | Scientific name | Herbal Part Used | Dynamics for Application |
| Lagundi | Vitex negundo Linn. | Leaves | Ingestion |
| Mariguso | Momordica charantia | Leaves | Ingestion |
| Oregano | Origanum vulgare | Leaves | Ingestion |
| Kantutay | Lantana camara | Leaves | Ingestion |
| | | | |

| Cyperus iria | Leaves | Ingestion | |
|-------------------|--|--|---|
| Hydrocotyle | Stem, | Ingestion | |
| sibthorpioides | roots | | |
| Euphorbia hirta | Leaves | Ingestion | |
| Senna alata | Leaves | Ingestion | |
| Mentha cordifolia | Stem | Ingestion | |
| | Cyperus iria Hydrocotyle sibthorpioides Euphorbia hirta Senna alata Mentha cordifolia | Cyperus iriaLeavesHydrocotyleStem,sibthorpioidesrootsEuphorbia hirtaLeavesSenna alataLeavesMentha cordifoliaStem | Cyperus iriaLeavesIngestionHydrocotyleStem,IngestionsibthorpioidesrootsEuphorbia hirtaLeavesIngestionSenna alataLeavesIngestionMentha cordifoliaStemIngestion |

No observation was conducted as folk healers narrated that herbs were only prescribed and not prepared by them for patients.

Design of the Experimental Procedure

Descriptive-quantitative approach was the overall design of the study. This was considered to numerically screen the saponin content of the identified herbs based from the results of trial testing. Herbs were collected, chopped, sundried, while others were decocted. Preparation of the herbs prior to the laboratory testing was based from the methods of preparation prescribed by the folk healers. This was done to mimic the practices narrated by folk healers, and to ensure the validity and consistency of the saponin content prior to application of the locals. Distinct herbal parts were also used like leaves, bark, and roots based from the recommended herbal parts by the folk healers. Table 2 shows the corresponding dynamics of preparation for every herb cited in the study.

Table 2. Dynamics of preparing the extracts from the cited herbs of the folk healers.

| Herbs (Local name) | Herbal Part Used | Dynamics for Preparation | |
|-----------------------|---------------------|--------------------------|--|
| Lagundi | Leaves | Chopped and Pounded | |
| Mariguso | Leaves | Chopped and Decocted | |
| Oregano | Leaves | Sun-dried and Pounded | |
| Kantutay | Leaves | Pounded | |
| Tawilis | Leaves | Sun-dried and Decocted | |
| Suro-suro | Stem | Decocted | |
| | Roots | Sun-dried and Decocted | |
| Tawa-tawa | Leaves | Pounded | |
| Kurukasitas | Leaves | Sun-dried and Pounded | |
| Herba buena | Stem | Chopped and Decocted | |

The extract yield from the varying dynamics of preparation was subjected to a modified Saponin Foam Test [28]. This was done by heating 5ml of the plant extract between 80 to 90°C and allowed to cool. Formation of foam 5 minutes after shaking the boiled extract indicates the presence of saponin. On the other hand, quantity of saponin was determined by the height of stable foam after 5 minutes. The experimentation was repeated in 3 trials to ensure consistency of results.

Materials

Herbal recommendations were collected at Cagrarav island. The identity and authenticity of the herbs were testified by the SciTech Innovations, Database of Biology. Herbal materials include 50 grams of leaves of lagundi, mariguso, oregano, kantutay, tawilis, tawa-tawa, and kurukasitas. Also, 50 grams of stem of suro-suro and herba buena, and 50 grams of suro-suro roots were used in the study.

For preparatory dynamics of the herbal extracts, materials used include 50 grams of herbal part, test tubes, beakers, 100ml of tap water, and bunsen burner for decoction. Tap water was used as it replicates the original material used for preparing the herbal extract. Moreover, preparation through chopping includes 50 grams of the herbal part and laboratory scalpels. For sun-drying, 50 grams of the herbal part was used and dried for 2 days until the plant material becomes semi-brittle. For pounding, mortar and pestle were used to isolate the extract from 50 grams of identified Folk medicines that were prepared in herbal part. combination of any of the preparatory dynamics used 50 grams only of the herbal part in a series of multiple preparatory practices.

For Saponin Foam Test, 9 test tubes were used, the herbal extract processed from the preparatory dynamics specified in Table 2, bunsen burner, thermometer to monitor temperature, test tube racks, and caliper to measure the height of foam. The same materials were used across all 3 trials. Nonetheless, herbal extracts were re-prepared prior to the beginning of all trials to determine the consistency of preparatory methods in measuring saponin content of extracts.

Data Analysis

Descriptive and inferential statistics were used to treat the data. Specifically, mean was used to determine the average saponin foam height of each herb across 3 trials. A t-test was used to compare the mean of saponin foam height which resulted from raw prescribed procedures vis-a-vis the eliminated extreme heat. Analysis of Variance (ANOVA) was utilized to test the significance of difference in the measure of saponin foam height across all herbs, and among trials. A post-hoc analysis through Bonferroni correction was also employed to back up the result of ANOVA testing and determine the data set that caused the significance in the difference of saponin foam height.

IV. RESULTS AND DISCUSSION

Herbs Used in Cagraray Island for Cough and Colds

Accounts from folk healers revealed a shortlist of herbal prescriptions used to treat cough and colds in the locale. Common to all interviewed folk healers were the prescriptions of herbs like lagundi, mariguso, oregano, kantutay, tawilis, suro-suro, tawa-tawa, kurukasitas, and herba buena (Figure 2). Leaves, stem, and roots were the herbal parts processed for treating cough and colds. Prior to ingestion of the extract, preparation dynamics were also recorded like that of chopping, pounding, sun-drying, and decocting. These preparations come in pairs depending on the herbal material used for the treatment (Table 2).





Lagundi (Vitex negundo Linn.)

Mariguso (Momordica charantia)



Oregano (Origanum vulgare)

Kantutay (Lantana camara)







Herba buena (Mentha cordifolia) Figure 2. Folk medicinal herbs prescribed by the folk healers of Cagraray island

Saponin Content of Herbal Prescriptions

Extract preparation was performed following the dynamics prescribed by the folk healers. Experimental set-up was designed in accordance to Saponin Foam Test where extracts were heated, cooled, and shaken to allow formation of stable foam for 5 minutes indicating Saponin presence.

It is important to note that the same quantity (50g) of herbal part was used in the decoction of herbs. Ranking the results showed high levels of saponin in *Kurukasitas* extract as supported by the mean. This is followed by *Oregano, Kantutay, Lagundi,* and *Tawa-tawa*. On the other hand, *Herba buena, Suro-suro, Mariguso,* and *Tawilis* contain low amount of Saponin. The use of 2-sample *t*-test (summarized data) revealed that the herbs with least saponin significantly differ from the aforesaid herbs with high saponin content (p<0.05). This statistical difference implies that the presumed effectiveness of two groups of herbs vary. Below (Table 3) is the summary of Saponin Foam Test results across all herbal extracts for treating cough and colds.

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Table 3. Phytochemical screening of saponin after shaking the cooled extracts in the following herbs for three trials.

| Herbal Extracts | Presence of foam after five (5) minutes. Indication of foam height (mm) if herbal extract showed foam. | | | Mean |
|-----------------|--|--------------|--------------|------|
| | 1st trial | 2nd trial | 3rd trial | |
| Kurukasitas | 5.6 | 5.3 | 5.5 | 5.47 |
| Oregano | 5.5 | 5.3 | 5.5 | 5.43 |
| Kantutay | 4.2 | 4.0 | 4.5 | 4.23 |
| Lagundi | 4.2 | 4.0 | 4.4 | 4.20 |
| Tawa-tawa | 3.5 | 3.5 | 3.8 | 3.60 |
| Herba buena | 1.2 | 1.0 | 1.0 | 1.07 |
| Suro-suro | 0.5 | 0.8 | 1.0 | 0.77 |
| Mariguso | 0.5 | 0.0 | 0.0 | 0.17 |
| Tawilis | 0.0 | 0.0 | 0.0 | 0.00 |

An intriguing pattern of similarity was observed in the data. Reviewing the dynamics for preparation showed that the identified herbs with least amount of saponin were processed through extreme heat (decoction). On the contrary, identified herbs with high levels of saponin were not treated with extreme heat (some of them were sundried), or never processed with heat. This assumption brought significant impact in the findings to test whether heat affects the degradation of saponin levels in the extract. To test this assumption, a 3-trial follow up of the experiment was re-conducted by eliminating all sources of extreme heat in the preparatory dynamics including the boiling of extract in the saponin foam testing. Table 4 showed the surprising result of the re-test.

| Table 4 . Phytochemical screening of saponin eliminating all |
|---|
| sources of extreme heat (decoction, and boiling in saponin |
| C · · · · · · · · · · · · · · · · · · · |

| foam testing). | | | | |
|-----------------|--|--------------|--------------|------|
| Herbal Extracts | Presence of foam after five (5) minutes. Indication of foam height (mm) if herbal extract showed foam. | | | Mean |
| | 1st trial | 2nd trial | 3rd trial | |
| Kurukasitas | 6.2 | 6.2 | 6.4 | 6.27 |
| Oregano | 6.0 | 5.8 | 6.2 | 6.00 |
| Lagundi | 5.3 | 5.0 | 4.4 | 4.90 |
| Tawa-tawa | 4.8 | 4.7 | 5.0 | 4.83 |
| Kantutay | 3.8 | 3.4 | 3.4 | 3.53 |
| Suro-suro | 2.5 | 2.3 | 2.6 | 2.47 |
| Herba buena | 2.3 | 2.5 | 2.3 | 2.37 |
| Tawilis | 2.1 | 2.3 | 2.3 | 2.23 |
| Mariguso | 1.4 | 1.3 | 1.6 | 1.43 |

To visualize the effect of extreme heat on the saponin content of herbal extracts, Figures 3 and 4 are presented.



Figure 3. Saponin content of herbal extracts following the prescribed method of its preparation using extreme heat (decoction and boiling in Saponin Foam Test).



Figure 4. Saponin content of herbal extracts eliminating all sources of extreme heat (decoction and boiling in Saponin Foam Test)

The figures revealed a vivid increase in the measure of saponin levels when heat is eliminated. This was supported by the result of t-test indicating a significant difference in the mean levels of saponin produced between raw prescribed procedures compared to the eliminated extreme heat in the process (p>0.05). Furthermore, the data connote that the methods employed by the locals on the usage of herbal medicines do not harness the optimal saponin content of the material as it degrades in the process of decoction. Therefore, only herbs processed without the intervention of extreme heat were deemed effective following the prescribed procedures by the folk healers. Nevertheless, further analysis need to be conducted if some active phytochemical constituents of the heated extracts remain stable that cooperates in the treatment of cough and colds.

Significance of Difference in the Measure of Saponin Foam Height Among Herbs and Trials.

To test the levels of saponin content and consistency of trials, ANOVA was executed using the data on saponin

foam height. This test was performed based from the original methods prescribed by folk healers integrating extreme heat in the processing of extracts. Table 5 shows the result of comparative analysis.

 Table 5. Comparative analysis of (a) herbs with high saponin content, and (b) consistency of trials.

| Divisions | ANOVA for saponin content | Post-hoc Analyis ^a (<i>Bonferroni</i>) | ANOVA for trials |
|---|---------------------------------|---|-------------------------------|
| High Saponin Content (<i>Oregano</i> , <i>Kantutay</i> , <i>Kurukasitas</i> , <i>Lagundi</i> , and <i>Tawa-tawa</i>) | 0.000 (Significant) | p<0.006 between (1) <i>Kurukasitas</i> and <i>Oregano</i> , and (2) <i>Kantutay</i> and <i>Lagundi</i> . (Not Significant) | 0.833 (Not Significant) |
| Low Saponin Content (<i>Herba</i> <i>buena, Suro-</i> <i>suro,</i> <i>Mariguso,</i> and <i>Tawilis</i>) | - | - | - |

^{*a*}Bonferroni alpha level at 0.006 with 8 comparative events ANOVA test determined the differences in the mean of results for herbs with high saponin content. The result revealed difference (p < 0.05) signifying that the saponin levels did vary. Looking at the mean, this shows that Kurukasitas significantly contains higher quantity of Saponin in its extracts as compared to its herbal counterparts. However, post-hoc analysis through Bonferroni correction revealed no statistical difference in the foam height between Kurukasitas compared to Oregano (p>0.006) demonstrating similarity in the screened saponin. Likewise, the post-hoc showed similarity (p>0.006) in the height of saponin foam between Kantutay and Lagundi. Nevetheless, saponin foam height among Kurukasitas and Oregano, Kantutay and Lagundi, and Tawa-tawa were statistically different (p<0.006) suggesting varying concentration levels of screened saponin.

To test the consistency of trials, another ANOVA was administered for difference. Result revealed that the trials did not vary in results as shown in statistical p value of 0.833. This further connotes that the results have been consistent and were not contaminated by external variances that could possibly alter screening results. This testifies for the credibility of the gathered data in the saponin testing.

Discussion

The screening of saponin was proven to exist in the identified folk medicinal herbs in the Cagraray island. This was proven to be theoretically and scientifically effective in treating cough and colds. Varying amounts of saponin was discovered in the extracts of the recommended herbs indicating that some of these herbal medicines can be used as an organic substitute treatment to commercialized medicines. This was related to an existing account where herbs were proven to treat or ease cough in a meta-analysis study [29]. Narratives from folk healers have previously testified for the efficacy of the suggested herbal treatment manifesting saponin as the fundamental phytochemical responsible for the expectorant and antitussive property. On the contrary, the result of the testing following the recommended procedures by folk healers revealed that saponin is degraded by the intervention of heat which could mean that other phytochemical constituents resistant to heat may have contributed in the relief of cough and colds. This supports the current findings on the reduced quantity of saponin when introduced to extreme heat temperatures [30]. The possibility of the hypothesis that other phytochemicals might be accountable for the alleviating effect was supported by other studies indicating that flavonoids [31], alkaloids [32], and tannins [33] were common phytochemicals that also exhibit antitussive and expectorant effects. Nonetheless, the current findings showed that the use of appropriate harnessing methods can properly utilize the optimal level of saponin to fully consider its potential for treating cough and colds.

V. CONCLUSION AND FUTURE SCOPE

The phytochemical screening of saponin revealed varying levels in the extract of prescribed folk medicines in the *Cagraray* island. This concludes that the herbal prescriptions of folk healers were truly effective in treating cough and colds, and were a good substitute to synthetic medicines. Furthermore, the current study reckons that years of practice and observation of folk healing remedies made a bridge between science and indigenous knowledge primarily aimed at sustaining the medicinal needs of the locals. Underlying scientific principles of the folk medicines used for treating cough and colds were proven and tested based from the saponin screening test. However, thorough understanding of the preparatory dynamics needs to be employed by the locals to absolutely utilize the phytochemical power of saponin.

As to future studies, a thorough phytochemical analysis needs to be conducted to isolate the antitussive and expectorant properties of the recommended herbs. Since extreme heat affects saponin quantity, a study needs to be carried on specific heat ranges that degrades saponin, and also heat ranges tolerable for saponin to remain stable. Also, *in vivo* assay needs to be experimented to truly test the antitussive and expectorant properties of these recommended herbs, which further requires a toxicity study on the effect of other phytochemicals on other animal tissues.

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AUTHOR'S PROFILE

Michael B. Bibon. Michael B. Bibon is a science and research teacher at Cawayan National High School, Bacacay, Albay, Department of Education-Albay Division, Philippines. He earned his Ph.D. at Bicol University Graduate School, Legazpi City. His interest lies in



culture-based studies, personality assessment, and phytopharmacology. He served as LAS writer in science and currently working as publisher of M. BIBON Publication. He has authored books particularly in IMRAD research paper development and published research papers in soft and hard sciences.