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Phytochemical Screening of Brugmansia versicolor Lagerheim Flower and Leaf Crude Extracts

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Abstract

The study determines the potential secondary metabolites of Brugmansia versicolor Lagerheim necessary in the medical field using phytochemical screening. The results revealed that the flowers and the mature leaves of the B. versicolor can be used in the medical discipline, particularly in pharmacology as antimicrobial, antidiarrheal, and anthelminthic. Relative to this, the psychoactive, specifically anticholinergic effect of the plant is more potent on the flowers as contrast to the leaves in terms of its contents.

Keywords: Brugmansia versicolor Lagerheim, Phytochemical screening.

INTRODUCTION

B. versicolor locally known as "Trompeta", mistakenly termed as "Talampunay" and known in the English language as Angel's Trumpet is a plant species contained within the nightshades family popular for having alkaloids such as solanine, tropane, nicotine, and capsaicin which comes in various shades of pink and yellow (Stuart Jr. 2016;Rätsch, 2005). The plant has a long history of being one of the most important shamanic plants but due to errors on ethnographic reports having misconceptions to that of *Brugmansiasuaveolens*and *Brugmansia x insignis*, there is only a minimal number of literatures pertaining to B. versicolor. The plant belongs to the Brugmansia genera identified for its alkaloids – tropane, atropine, scopolamine, and hyoscyamine, which are well-known for their anticholinergic effect (Yadav et al., 2016; Yarnell, 2015; Bounty et al., 2013; Das et al., 2010; Geller, 2010). Their genus, the Brugmansia is second to that of Atropa belladonna for having the highest potency of anticholinergic effect. This was the reason why shamans use the genus for rituals – it gives hallucinogenic effect that the shaman termed as divination (Stuart Jr. 2016; Bounty et al., 2013;Geller, 2010). In the long run, ethnopharmacological application of this genus was cited in Geller's work, as well as Dr. Stuart Jr., who goes along with the idea and specified the Brugmansia's uses as to spasmolytic, antiamoebic, anti-fungal, and antioxidant.

In the year 2015, a journalist wrote an article on the drug burandanga which was also known as scopolamine. The drug was said to zombify victims – removes a person's

free will, to assault victims, or rob them. Incidents pertaining to this drug increased making the urban legend existent in the areas where Brugmansia genus is endemic (Saner, 2015). This might likewise happen in the country due to the alarming issues on the Brugmansia species (Brugmansia spp.) used as an alternative drug. War on abused drugs in the Philippines was the trend on our society due to the mandates of the former President Rodrigo R. Duterte. Relative to this, there is a significant result on the increase of drug pushers and users caught and rehabilitated. Howbeit, the probability of the plant to become an alternative for narcotics such as marijuana and shabu is considerably high. Angel's Trumpet species became popular in far-flung areas of the country as common alternatives for smoking and narcotics. Reports of GMA News Online in 2008 revealed that youth, specifically boys, use the leaves of these plants as an alternative for the commercial and traditional cigarettes because its effect is said to be greater than all other drugs. This archaic practice is deemed to be faulty because of the lack of information about the deleterious effects of the plants to humans. In Benguet, it is a practice on asthmatic individuals to smoke on the leaves of the *B. suaveolens* (Pennachio et al., 2010). It has a beneficial effect when inhaled in lower dosage but when abused, the effect can be detrimental consequential to its relation on the direct effect of the plant species to the central nervous system. Higher dosage will result to delirium, extreme thirst, pupil dilation, vision impairment, and hallucinations. High temperature, flushed skin, and palpitations may be observed. According to Stuart (2016), on non-fatal cases, visual impairment and lethargy may happen for quite some time. For those who have lethal incidents, GMA News Online (2008) stated that convulsions, coma, and even death will be the end case. Regarding this issue, Stuart Jr. (2016), Geller (2010), and Bounty et al. (2013) asserted that everything about Brugmansia spp. are poisonous to both human and animals which led to its use in sorcery and black magic for poisoning in centuries.

The purpose of this project is to identify the secondary metabolites of the B. versicolor flower (bud, mature, and young) and mature leaf crude extracts with which comparing and contrasting them to one another will be of necessary to further elucidate their similarities and differences. It also intends to determine the pharmacological uses of these identified secondary metabolites. Herewith, identifying the bioactive constituents of the B. versicolor can open avenues as to the significant information regarding the Peach Angel's Trumpet as to why this plant and all other species of Brugmansia are used as alternatives for narcotics. It may also get the attention of the government against the abusive use of the people especially the youth on the plant. And this output can be the steppingstone into the furtherance of medical research on the uses of the B. versicolor species.

Objectives of the Study

1. Determine the secondary metabolites found in *Brugmansia versicolor*Lagerh*.* flower and leaf crude extracts;

2. Compare and contrast the secondary metabolites found in *Brugmansia versicolor* Lagerh.in terms of:

2.1. bud flower crude extracts;
2.2. young flower crude extracts;
2.3. mature flower crude extracts;
2.4. mature leaf crude extracts;

3. Identify medical applications of the traced secondary metabolites in *Brugmansia versicolor* Lagerh. Flower and leaf crude extracts.

METHODOLOGY

Extraction Process

The researcher gathered samples for each variety of the plant species parts namely bud, young, and matured flowers, and matured leaves in Purok 4, Capirpiriwan, Cordon, Isabela. The samples were grouped and washed with running water. The samples were grouped accordingly. Through the natural process of drying, the grouped samples were air-dried for two weeks (336 hours) in a room. This is done in order to minimize the loss of compounds especially those of the volatile components. Grinding is the initial process corollary to extraction. This is done to minimize the surface area of the samples leading to an effective extraction. After grinding, the specimens were placed in a beaker and labeled correspondingly. An approximated 100 mL ethanol was poured on each sample. It was covered with a paper and sealed through rubber bands. After soaking the specimens for 24 hours in the ethanol solution, the specimens were filtered in cotton placed in a funnel. It was set aside for two days to separate the potential secondary metabolites from the ethanol by means of evaporation. In order to catalyze the evaporation process, the researcher opted to use the water bath maintaining a temperature of 40-50 degree Celsius. The remains were placed in vials with the corresponding labels for each sample.

Phytochemical Screening

The researcher divided the Thin Layer Chromatography (TLC) Silica Gel Plate into four equal parts having same measurements (height: 6 cm; width: 5 cm; solvent front: 0.5 cm; spot line: 1cm). She then prepared the necessary materials for the spotting of samples on the prepared TLC silica gel plates. She allotted one centimeter per sample

(S1: bud; S2: young; S3: mature; S4: leaves). She used a capillary tube to get the samples and consequently washed it with methanol one after the other. The researcher then placed the prepared TLC plate on the developing chamber with the 20-milliliter developing solvent of Ethyl acetate and Methanol (8:2). After the developing solvent reached the solvent front, the plate was dried for about two minutes. Then, the researcher dipped the plate on the reagents she opted to use to test the components of the samples.

Test Outcome Determinants

• Phenols/Tannins/Flavonoids

- Blue spots were formed upon dipping the prepared plate on the Potassium Ferricyanide – Ferric Chloride.

• Triterpenes/Steroids/Essential Oils

- Triterpenes and sterols appeared mainly as blue-violet spots after dipping the prepared plates on the Vanillin-sulfuric acid reagent. Essential oils formed zones with a wide range of colors.

• Alkaloids

- After dipping the prepared TLC silica gel plate on the Dragendorff's reagent, there were brown-orange visible spots.

RESULTS AND DISCUSSIONS

Phytochemical Screening of Brugmansia versicolorLagerh. Samples on Secondary Metabolites Using Preliminary Reagent

Although bubbles were formed, there were still traces of potential secondary metabolites on the TLC plate due to the different spots formed after the plate was heated. This means that the samples vary on the number and concentration of their secondary metabolites. The leaf crude extracts produce various shades and colors as compared to the flower crude extracts which produced minimal colors. This means that the leaf crude extract contains a higher number of secondary metabolites in relation to that of the flower crude extracts.

Phytochemical Screening of Brugmansia versicolorLagerh. Samples on Secondary Metabolites Using Potassium Ferricyanide – Ferric Chloride Reagent

Blue spots were evident in every sample which signifies the presence of phenols/tannins/flavonoids on every sample. Albeit generalized, the findings were supported by San Luis et al. about the *B. suaveolens* flower and leaf extracts having flavonoids and tannins as their secondary metabolites. Flavonoids have been known to exhibit many biological activities such as antimicrobial where it binds to adhesins of bacteria; antidiarrheal of which it acts as inhibitor for the release of autocoids and

prostaglandins, as well as the contractions caused by spasmogens, and the gastrointestinal release of acetylcholine. It stimulates normalization of the deranged water transport across the mucosal cells. It also acts as photo receptors, feeding repellents, but most studies focused on the flavonoids' ability as antioxidant (Saner 2015; Stuart Jr., 2016). Moreover, Guevara et. al stated that flavonoids include anthocyanins and the leucoanthocyanins, other types of flavonoids consist of cathechins, aurones, chalcones.

Tannins and the phenols or polyphenols on the other hand, are groups of secondary metabolites which show antimicrobial activity where it binds to adhesins, these groups also act as enzyme inhibition, substrate deprivation, membrane disruption, and metal ion complexation. They have an antidiarrheal effect as well which makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea. Having anthelminthic properties, these groups of metabolites increase supply of digestible proteins by animals forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G.I. metabolism (Saner 2015; Stuart Jr., 2016). Tannins also have the ability to react with the precipitate proteins forming stable water-insoluble copolymers. They have astringent action as well which makes it popular in the medical fields (Guevarra, 2004).

Phytochemical Screening of Brugmansia versicolorLagerh. Samples on Secondary Metabolites Using Vanillin-sulphuric Acid Reagent

Traces of yellow color on the fourth sample (leaf crude extract) were evident which corresponds to the presence of terpenes/steroids/essential oils on the sample. Howbeit, it was in contrast to that of the work of San Luis et al. (2014) having *B. suaveolens* flower extract as the sample which contains steroids. Terpenes or terpenoids and essential oils are antimicrobial in a way that it disrupts the bacterial membrane and antidiarrheal for it inhibits the release of autocoids and prostaglandins similar to the effect of flavonoids and alkaloids. Steroids are groups of secondary metabolites which have antidiarrheal effect. This group enhances intestinal absorption of sodium and water (Tiwari, 2011).

Phytochemical Screening of Brugmansia versicolorLagerh. Samples on Secondary Metabolites Using Dragendorff's Reagent

Although a paler orange was noted on the colors of the flower crude extracts likened to the leaf crude extract which has a more intense orange color, all samples showed traces of alkaloids. The result was reinforced by the findings of San Luis et al. (2014) of the *Brugmansiasuaveolens* flower and leaf extracts. Alkaloids are one of the groups of secondary metabolites which acts as antimicrobial. It intercalates into cell

wall and DNA of parasites. It also inhibits release of autocoids and prostaglandins which is similar mechanism to that of the flavonoids. The alkaloid group possesses anti-oxidating effects, thus reduces nitrate generation, which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, and acts on Central Nervous System causing paralysis.

This makes the group antidiarrheal and anthelmintic. From the citations of Yadav, the alkaloid compounds can be attributed to the family where the species is a part of. They are known to have anticholinergic effects due to their tropane alkaloid content.

Table 1. Relative Flow Rate of the Identified Secondary Metabolites of

*Brugmansia versicolor*Lagerh. Flower and Leaf Crude Extracts

Sample	**Relative Flow Rate (mm)**		
	Phenols/Flavonoids/Tannins	Terpenes/Steroids/ Essential Oils	Alkaloids
1 (Bud FlowerExtract)	0.24		0.38
2 (Young Flower Extract)	0.23 0.21		 0.39
3 (Mature Flower Extract)	0.17		0.39
4 (Leaf Extract)	0.48	0.038	0.27

As presented in Table 1, all the samples contain phenols/flavonoids/tannins. The young flower crude extract was noted to have two secondary metabolite compounds almost alike to that of the bud flower extract due to their similar relative flow rate. On the other hand, the mature flower extract contains the least score of Rf value. This means that it has polar compounds compared to that of the other samples. Corollary to this, the leaf crude extract, was observed to have the highest Rf value, this implied that almost all the compounds present in the sample is nonpolar. It is also the only sample which yielded a positive effect on the vanillin reagent having a 0.038 Rf value. This signified that the leaf sample of *B. versicolor* was confirmed to have one of the following compounds: terpenes, steroids, or essential oils. All the samples appeared to have alkaloids as their secondary metabolite as well. Moreover, the flower crude extracts were comparable with their alkaloid contents which meant that they may have the same type of alkaloid. The flower samples of B. versicolor set the highest comparable scores relative to the alkaloid content of its leaf sample.

Summing up all the components of each sample, bud, young, and mature flower crude extracts are positive on having phenols/flavonoids/tannins and alkaloids as its secondary metabolite. Whereas the leaf crude extract was positive in all tests containing phenols/flavonoids/tannins, terpenes/steroids/essential oils, and alkaloids.

CONCLUSIONS

Based on the obtained results of the project, it was concluded that the flowers – bud, young, and mature, and leaves of B. versicolor, can be used by medical practitioners in the fields of pharmacology as potential sources of pharmaceuticals. Although all parts are good sources of the secondary metabolites, the mature leaves have the highest potential as sources of more compounds while the flowers are the best sources of alkaloids.

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