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Determination of secondary metabolites and antibacterial property of extract from the leaves of *Stachytarpheta jamaicensis* (L.) Vahl

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Abstract

The study evaluated the phytochemical profile and antibacterial activity of the ethanolic extract of *Stachytarpheta jamaicensis* (L.) Vahl. leaves to validate the folkloric claim as an external anti-bacterial agent using the crude extract obtained by macerating the powdered dried leaves with 95% ethanol for 72 hours, and concentrated in vacuo. Phytochemical screening using the standard qualitative technique revealed the presence of flavonoids, phenolics, saponins, tannins and terpenoids. Paper disc diffusion method was applied in evaluating the antibacterial activity of the ethanolic extract against a common bacterium, *Staphylococcus aureus*. The activity determination resulted to zone of inhibition between 5 - 26 millimeters. The results indicate that the leaf extract has a potential activity against the test organism and could be attributed to the presence of the secondary metabolites indicated.

Keywords: Antibacterial activity, secondary metabolites, ethanolic extract, phytochemical screening

1. Introduction

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents; search for plant products has increased for their potential antimicrobial activity.

The usage of herbal plants as traditional health remedy is the most popular for 80% of the world population in Asia, Latin America and Africa. These plants were also reported to have minimal side effects (Doughari, 2006) [1]. Concurrently many people in developed countries have begun to turn to alternative or complementary therapies including medicinal herbs (Fransworth and Soejarto, 1991) [2]. In India, medicinal plants are widely well used by all sections of people either directly as folk remedies or in different indigenous system of medicine or indirectly in the pharmaceutical preparations.

Stachytarpheta jamaicensis, (*S. jamaicensis*) commonly known in the Philippines as kandikandilaan belongs to the family Verbenaceae. A well branched herb 2-3 ft, high with very long narrow spikes, flowers are deep blue with white centre; a weed and characteristically drought tolerant. The plant has been used locally as an abortifacient and in the management of asthma, headache, bronchitis, and bruise. In northern Nigeria a decoction of the leaves with natron is given for dysentery in humans and for similar conditions in horses (Burkill, 1985) [3]. This study is an attempt to determine the secondary metabolites present and to evaluate the antibacterial activity of the leaf extract of *S. jamaicensis* against *Staphylococcus aureus* (*S. aureus*), a pathogenic bacteria associated with common skin disease.

Materials and Methods

Plant material

Plant samples of *Stachytarpheta jamaicensis* (L.) Vahl. were collected from the vicinity of the Isabela State University Echague campus, Echague, Isabela, Philippines. The identity of the plant was authenticated by a forester from the Community Environment and Natural Resources Office, San Isidro, Isabela, Philippines. One voucher specimen was deposited under the number S5TBZEch24/2/15.

Extraction of active constituents

Whole plants were collected from the field. The leaves were isolated and were washed, cut into small pieces and air dried for three days resulting to coarse particles. A 500 gram air dried sample was macerated with enough quantity (1.650 L) of ethanol (95%). The sample was soak undisturbed at room temperature for 72 hours, and then filtered. Extraction was repeated using 1.0L for another 48 hours. The filtrates were combined and, concentrated under reduced pressure using a rotary evaporator at a temperature below 50 °C. The crude extract was further dried to a constant weight of 23.40 g (4.68%).

Phytochemical evaluation of the crude extract

Chemical classes of secondary metabolites were investigated in the leaf extract of *S. jamaicensis* through identification reactions using standard procedures as stated: alkaloids with Mayer's and Dragendorff's reagents (Farnsworth, 1966; Harborne, 1998) [4, 5], flavonoids with the use of Mg and HCl (Silva *et al.*, 1993; Houghton and Raman, 1998) [6, 7], tannins with 1% gelatin and 5% ferric chloride solution, and saponins with ability to produce suds (Houghton and Raman, 1998) [7]. Liebermann- Bucharid test consisting of a mixture of glacial acetic acid and sulphuric acid (19:1) was used to differentiate the types of triterpenoids and steroidal nuclei present (Guevara, 2005) [8] and Borntrager's test using Ammoniacal solution to indicate the presence of anthraquinones (Farnsworth, 1966) [4].

Detection of Antibacterial Activity

The antibacterial activity of the different plant extract was evaluated against the reference strain *S. aureus*, a gram positive organism. The disc diffusion method (Guevara, 2005) [8] was used in the microbial evaluation. Bacterial cultures maintained on nutrient agar slants were taken and aseptically inoculated into 10 ml of sterile broth. Then broth containing the bacteria were incubated at 37 °C for 24 hours, and designated as the working stocks used for antibacterial studies. *S. jamaicensis* leaf extract was screened over the range of 0.5 to 10 mg/ml concentration using paper disc diffusion method. About 1ml of the bacterial suspension was taken and diluted in 10 ml autoclaved water and this suspension was inoculated on semi - solidified nutrient agar medium. Small autoclaved discs about 6 mm diameter size of Whatmann filter paper (No.41) were impregnated with 1ml solution of the different concentrations of the extract then these saturated paper discs were inoculated equidistantly. These set up were incubated at 37°C for 24 hours. In the whole investigation, paper disc impregnated with 85% ethanol was taken as control. The zone of inhibition (ZOI) around each disc indicative of the sensitivity at that concentration was observed and measured using a sterilized micro caliper. Activity of the extract was compared with the corresponding references (Guevara, 2005) [8]:

ZOI value of <10 mm, maybe expressed as inactive

ZOI value of 10-13 mm, partially active

ZOI value of 14-19 mm, active

ZOI value of > 19 mm, very active

Results and Discussion

The preliminary phytochemical screening of the leaves of *Stachytapheta jamaicensis* indicates that the plant is quite rich in tannins, saponins and phenolics. Moderate concentration of triterpenes and flavonoids were indicated by the result of qualitative tests on the leaf extract. On the other hand alkaloids, glycosides, steroids and anthraquinones were conspicuously absent (table 1).

Table 1: Phytochemical screening of the ethanol extracts of *S. jamaicensis*.

Phytochemical	Reaction
Alkaloid	-
Anthraquinones	-
Flavonoids	++
Glycosides	-
Phenolic groups	+++
Saponins	+++
Tannins	+++
Terpenoids	++

Legend: + Presence of the phytochemical, +++ = High;

++ = Moderate; + = Low

-Absence of the phytochemical evaluated

Saponins indicated to be present in the leaf extract are characterized by their surface-active properties and they dissolve in water to form foamy solutions. Because of surface activity, some drugs containing saponins have a very long history of usage. Saponins have been implicated as a bioactive antibacterial agent of plants containing them.

Phenolic compounds were found to be present in high quantity in the extract have been associated with antioxidant activities and were earlier reported to have some antibacterial activities (Tomas-Barberan *et al.*, 1990)^[9], and might have complimented or potentiated the saponins in the antibacterial activities exhibited by the extract. The exhibited antibacterial properties of *S. jamaicensis* could be attributed to the presence of saponins and phenolics in the plant.

Tannins are water-soluble polyphenols that are commonly found in higher herbaceous and woody plants. Tannins have been reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* (Chung, K.-T., Wong, T. Y., Wei, C.-I., Huang, Y.-W. & Lin, Y., 1998) [10]. Tannins form chelates with metal ions and are therefore different from smaller phenols. The antimicrobial mechanisms of tannins summarized by (Havsteen B., 1983) [11] as follows: (i) the astringent property of the tannin may induce complexation with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when mixed with tannins. (ii) A tannin's toxicity may be related to its action on the membranes of the microorganisms. (iii) Complexation of metal ions by tannins may account for tannin toxicity.

On the other hand, flavonoids, are known to be a class of natural products of high pharmacological potency. These compounds are ubiquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey (Havsteen, 1983) [11]. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases.

The antibacterial activity of flavonoids is being increasingly documented. Crude extracts from plants with a history of use in folk medicine have been screened in vitro for antibacterial activity by many research groups. Flavonoid rich plant extracts from species of *Hypericum Capsella* (Dall'Agno R, Ferraz A, Bernardi AP, *et al.*, 2003) [12], and *Chromolaena* (El-Abyad MS, Morsi NM, Zaki DA, Shaaban MT, 1990) [13], have been reported to possess antibacterial activity which conforms with the result of this study.

The results of the antibacterial activity of the ethanolic extract of *S. jamaicensis* against *S. aureus* are presented in table 2. As indicated by the results, the inhibition zone against *S. aureus* ranged from 5 to 26 mm. The discs impregnated with concentration of 10mg/ml gave the highest zone of inhibition

considered to be active when compared with the standard (Guevara, 2005) [8]. The result is similar to data obtained by (Enwuru *et al.*, 2008) [14] when the antimicrobial activity of the whole plant of *Stachytarpheta indica* was evaluated.

Table 2: Inhibition of *Staphylococcus aureus* by ethanolic extract of leaves *Stachytarpheta jamaicensis*.

Concentration (mg/ml)	0.5	1	2	4	8	10
Zone of inhibition (Average)	5	8	12	15	18	26
Zone of inhibition of 85% ethanol, mm	8					
Size of disc, mm	6					

The present investigation reveals the antibacterial nature of this plant and these result justifies the folkloric claim of the community and that the plant could be exploited in the management of diseases caused by bacteria in human. Further studies to isolate and characterize the bioactive constituents of the plant extract are suggested. It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful drugs.

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