IJBPAS, June, 2018, 7(6): 1165-1172

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICALS AND BIOACTIVITIES OF TALAHIB (*Saccharum spontaneum*) ROOTS EXTRACT

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Received 15th Feb. 2018; Revised 14th March. 2018; Accepted 10th April 2018; Available online 1st June 2018

DOI: https://doi.org/10.31032/IJBPAS/2018/7.6.4480

ABSTRACT

This paper reported the phytochemical constituents and biological activities of talahib (*Saccharum spontaneum*) roots. Phytochemical screening revealed that *S. spontaneum* roots contain phenols, triterpenes, essential oils, steroids, anthraquinones, coumarin, anthrones, flavonoids, and alkaloids. The antioxidant and antibacterial activities of the ethanol extract were also determined. It was found out that the ethanol extract exhibited 68.97% scavenging activity against 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals. However, the extract did not show inhibitory activity against *Pseudomonas aeruginosa*. Thus, *S. spontaneum* roots could be a source of bioactive compounds with biological activities such as antioxidant.

Keywords: Phytochemical screening, antioxidant, *Pseudomonas aeruginosa*

INTRODUCTION

Saccharum spontaneum, commonly known as talahib, is a type of perennial grass that is coarse and erect. It grows up to 3.5 m and has linear leaves that are about 1 m long and 6 to 15 mm wide. In the Philippines, root decoction is used as a diuretic and for fever. In other countries such as in Bengal, *S. spontaneum* roots are used as galactagogue **[1]**. *S. spontaneum* exhibits antioxidative **[2]**, anti-diarrhoeal, and CNS depressant properties **[3]**, and it could also be used in cellulose production **[4]**. Its roots are also

used as astringent, emollient, refrigerant, diuretic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles and sexual weakness, it also contained minerals, organic acids, flavonoids and phenolic compounds which was found to possess antioxidant, mast cells calming effects, the roots are also used as food or parts of food that could deliver medical health aids including action and prevention of illnesses **[5]**.

Phytochemicals are bioactive compounds found in plants or plant-based products. These compounds are reported to have numerous of biological activities depending on the type of phytochemicals present. Some of these activities include insecticidal, phytotoxic, fungitoxic, nematocidal, antibacterial, antioxidant activities, some are for the plants defensive mechanism against herbivores and some are for the plants own growth, reproduction and responses to several abiotic and biotic stresses **[6, 7, 8, 9, 10, 11, 12]**. These phytochemicals can be extracted and screened at various methods for pharmacological investigation of certain plants.

Antioxidants, on the other hand, are substances that are found in plants. They could inhibit oxidation and remove potentially damaging effect of oxidizing agents in a living cell or organism. Antioxidants could prevent various human diseases like Alzheimer's disease, atherosclerosis, adult respiratory distress syndrome, cancer, and diabetes **[13].** DPPH assay is a much more stable method compared to the other methods. It is an easier and faster way to determine the antioxidant activity of a compound or plant extract. Unlike other methods, sample polarity is not necessary for the exhibited activity using DPPH method **[14]**.

This study reported the phytochemical constituents and biological activities of *S. spontaneum* roots, specifically, the radical scavenging activity and the antibacterial activity against *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Collection of *S. spontaneum* Roots

The root samples of *S. spontaneum* were collected from Bagong Sikat, Science City of Muñoz, Nueva Ecija, Philippines. The collected plant roots were washed with tap water and were air dried in room temperature for 3 weeks. After drying, the air dried roots were then pulverized using a blender.

Preparation of Ethanolic Extract

The pulverized root sample (20 g) was soaked in 95% ethanol for 48 hours. Then the extract was filtered using Whatman filter

paper no. 1. The filtrates were concentrated in rotary evaporator at 40˚C until dryness.

Phytochemical Screening

Phytochemical screening was carried out to detect secondary metabolites present. The plant extract was spotted on marked and labeled TLC (Thin Layer Chromatography) 7 x 4 cm, and was developed in acetate-methanol (7:3) mixture in the developing chamber. The spots for certain metabolite were visualized on the TLC plates and were exposed under UV light and hot plate to check the separation of the different compounds.

For typical visualization of secondary metabolites, vanillin-sulfuric acid reagents were used. This solution showed the presence of phenols, sterols, triterpenes, and essential oils. Methanolic potassium hydroxide was used to test anthraquinones, coumarins and anthranones while phenolic compounds and tannins were detected through the use of potassium ferricyanide-ferric chloride reagent. Dragendorff's reagent was utilized to spot alkaloids and antimony (III) was used to detect the presence of flavonoids **[15]**.

DPPH Radical Scavenging Assay

The concentrated extract was used to make a stock solution and aliquot was taken to make 1000 ppm dilution and 1000 ppm of catechin as control (1mg/mL). One millilitre of prepared stock solution was mixed with four mL of 0.1 mM DPPH solution in separate plastic cuvette. Reactions were done in triplicate. The prepared mixtures were incubated in the dark at 37ºC for 30 minutes. The absorbance readings were monitored at 517 nm using a UV VIS spectrophotometer. A lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical scavenging activities were compared to the activity of the control catechin. The ability to scavenge the DPPH radical was calculated using the formula: (A0-A1)/A0] × 100, where A0 was the absorbance of the control which is the DPPH without the test sample and A1 was the absorbance of the test sample containing the mixture of the DPPH and the sample. Catechin was used as the positive control **[16]**.

Antibacterial Assay

A bacterial suspension of *Pseudomonas aeruginosa* was provided by Immaculate Concepion Medical Center and was sub-cultured into a fresh nutrient broth. The bacterial suspension was adjusted to 0.5 McFarland standard and measured using a turbidometer. A sterile cotton swab was dipped into the bacterial suspension and aseptically spread onto the surface of the plated nutrient agar. After swabbing, blot-

dried paper discs (6mm diameter) previously soaked in the different treatments namely; *S. spontaneum* root extract, streptomycin (positive control), and 95% ethanol (negative control) were placed equidistantly on the media with bacteria. Plates were incubated at room temperature and the diameter zone of inhibition, if any, was measured after 24 hours.

RESULTS AND DISCUSSION

Phytochemical Composition

Phytochemical screening using TLC spot method of the ethanolic extract of *S. spontaneum* roots revealed the presence of essential oils, phenols, triterpenes, steroids, anthraquinones, coumarins, anthrones, flavonoids and alkaloids (Table 1). Similarly, these phytochemicals were also detected in the study of Khalid and Siddiqui **[5]**, along with the additional presence of carbohydrates, tannins, and terpenoids. Sathya and Kokilavani **[17]** reported the qualitative analysis of phytochemicals in the root extract of *S. spontaneum* using different types of solvents, and they found out that methanolic and ethanolic root extracts of the plant showed more phytochemicals, specifically the presence of tannins, steroids and glycosides.

Most of these phytochemicals have medicinal properties. Edeoga and Enata **[18]** reported that alkaloids were powerful pain reliever and topical anaesthetic in ophthalmology. Flavonoids, on the other hand, were reported by Woznicka *et al.* **[19]** to exhibit cardio-protection, anticancer, and anti-inflammatory properties. Anthraquinones shows potential anti-cancer and theraputic properties **[20]**. Triterpenes have potential in combating immune diseases **[21]**, while coumarins have anticoagulant properties and are potent anti-inflammatory drugs **[22]**.

Table 1: Phytochemical constituents of *S. spontaneum* roots

Phytochemicals	*S. spontaneum roots*
Essential oils	Present
Phenols	Present
Triterpenes	Present
Steroids	Present
Anthraquinones	Present
Coumarins	Present
Anthrones	Present
Flavonoids	Present
Alkaloids	Present

DPPH Radical Scavenging Assay

Antioxidants are substances that inhibit oxidation that causes several physiologic diseases. DPPH assay is one of the methods used in determining the antioxidant properties of compounds or substances. This study determined the radical scavenging activity of *S. spontaneum* root extract and the result is shown in Table 2. It can be seen that the radical scavenging activity of the root extract (68.97%) was higher compared to the radical scavenging activityof catechin (67.37%). Similarly, Sylvie *et al.* **[23]** reported that the three extracts of plants; *Acalypha racemosa, Garcinia lucida* and *Hymeno cardialyrata* have higher scavenging activity when compared to catechin. In the contrary, Khalid *et al.* **[2]** stated that the RSA of ethanolic extract of *S. spontaneum* roots was significantly lower than ascorbic acid, but found comparable to each other. In addition, Ripa *et al.* **[24]** showed that crude chloroform extract of *S. spontaneum* flower revealed antioxidant activity with the IC50 value of 43.04µg/ml.

In this study, anthrones, flavonoids, and phenols were detected in the roots. Yen *et al.* **[25]** reported that anthrone exhibited antioxidant activity by inhibition of peroxidation of linoleic acid and revealed that strong activity exposed by anthrone can be associated with its reducing power and scavenging effects on hydroxyl radicals. However, flavonoids have antioxidant and antiproliferative effect especially in metabolic systems **[26]**. Moreover, powerful antioxidant activities of phenolic compounds of olive oil were reported by Visioli *et al.* **[27]**. The presence of these three phytochemicals could make the ethanolic root extract of *S. spontaneum* a good antioxidant drug.

Table 2. Radical scavenging activity of root extract of *S. spontaneum*

Treatment	Radical Scavenging Activity (%)
S. spontaneum roots	**68.97**
Cathechin (control)	**67.37**

Antibacterial Assay

The ethanolic extract of *S. spontaneum* roots was also assayed for its antibacterial activity against *P. aeruginosa.* Based on the result, the extract did not show any inhibitory activity against the bacterial pathogen used. Similarly, Lapuz *et al*. **[28]** revealed that the crude ethanolic extract of *S. spontaneum* also did not inhibit the growth of *P. aeruginosa* using the four concentrations (0.5%, 1.0%, 1.5% and 2.0%). However, methanolic crude extract of *S. spontaneum* (150mg/ml) inhibited the growth of *P. aeruginosa* with 15.20 mm diameter zone of inhibition (Hussain *et al*., 2014). These findings strongly suggest that the anti-*P. aeruginosa*

effect of *S. spontaneum* root is solvent dependent. Steroids and essential oil which are found present in *S. spontaneum* roots are known for their antibacterial potential. Steroids exhibited antibacterial activity with a minimal inhibitory concentration of 32 µg/ml against *Pasteurella multocida* and *Staphylococcus aureus* **[11]**. Moreover, essential oils have means of action which includes several targets in bacterial cell **[12]**. Thus, the negative inhibitory effect of the extract could be explained by low amount of these two phytochemicals or could be due to the strong resistance of the tested bacterial pathogen.

CONCLUSION

Based on the significant results, it can be therefore concluded that *S. spontaneum* roots contain active phytochemicals such as essential oils, phenols, triterpenes, steroids, anthraquinones, coumarins, anthrones, flavonoids and alkaloids, which play significant roles on the biological activities such as antioxidant.

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