

Isolation of Solanine from Potato Leaves and Evaluation of Its Antimicrobial Activity

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Abstract: Herbal drug technology has become very important as they are considered to have very less or no side effects and toxic effects. Solanine is an alkaloid toxin found in members of the nightshade family, such as eggplants, potatoes, tomatoes, and the infamous deadly nightshades or belladonna. The objective of paper is to discover new possibilities of using traditional plant with proper chemical and pharmacological profile. The isolation of solanine from leaves of potato plant was done by authenticated process; dried leaves were used for the activity. The evaluation of solanine was done by standard chemical tests. The later process performed was thin layer chromatography which was performed by using chloroform: Menthol: Ammonia (7:3:0.5) solvent system for the analysis of glycoalkaloid solanine. IR graph of solanine resembling various peaks was obtained. Antimicrobial activity of solanine was tested on Gram-positive bacteria cultures prepared in nutrient agar medium. Ciprofloxacin was used as a standard drug. The antimicrobial activities of solanine were examined against bacillus subtilis by cup plate method. The solanine showed effective inhibition against the bacillus subtilis and also against staphylococcus aureus.

Keywords: isolation, antimicrobial, solanine

Plant Profile: Solanum Tuberosum L

Sunonyms: POTATO, Ma ling Shu

Scientific names	Common Names
Solanum tuberosum Linn	Patatas (C. Bis., Bik, Tag)
Solanum esculentum Neck	Potato (Engl)
Solanum aracatscha Bess	Ma ling Shu (Chin)
Lycopersicon tuberosum (L) Mill	Tu dou (Chin)

Botany

Patatas is a perennial herb with rough, pinnate leaves. Flowers are rather large, white or purple, star-shaped, and borne on compound inflorescences, 3 to 4 centimeters in diameter. Roots grow round and edible fleshy tubers.

Distribution

- Cultivated at higher altitudes, especially in the Mountain Province, Luzon, and in the Lanao region in Mindanao.
- Introduced from America.

Constituents

Study yielded a glycol-alkaloid, solanine,

- 1) In fresh plant ranging from 0.0101 to 0.0489%
- 2) In flowers ranging from 0.6 to 0.7%
- 3) In unripe fruit about 1%.
- 4) In seeds, 0.25%
- 5) Tubers and buns, 0.02%
- 6) In skin, 0.07% & starchy region, 0.002%.
- 7) In shoots, ranging from 0.02 to 0.05%.

Properties

- Sprouting growing tubers are considered poisonous, as well as the flowers, unripe seeds, and leaves as they

contain solanine. The full-grown tuber does not contain solanine.

- Study isolated putrescine N-methyltransferase, a calystegine, a nortropane alkaloid with glycosidase inhibitory activity.
- Antiscorbutic, aperients, diuretic, galactagogue, stimulant, emollient, antidote, antispasmodic.
- Considered a nervous sedative and stimulant in gout.
- Leaves believed to be poisonous.

Uses: Nutritional

- Very high starch content; valuable as an energy giving food.
- Potatoes grown in Baguio are deficient in calcium, and only fair sources of iron.
- Good source of fiber, vitamins B and C and minerals.
- Peels are high in potassium.
- Gently laxative, but non-purging
- Promotes milk
- Useful for gout.
- Potato peels tea for hypertension.
- Poultice of leaves as a tonic
- Used for scurvy, dyspepsia, hyperacidity, gout and arthritis.
- Decoction of leaves for chronic cough.
- Potato, ground to a paste, applied as a plaster to burns caused by fire.
- Poultice of grated raw potato used for light burns, arthritis, itching etc.
- Boiled potatoes used as emollient poultices.
- Used as antidote to poisoning by iodine.
- Extract of leaves used as an antispasmodic in chronic coughs, producing opium-like effects.
- Extract also used as narcotic.

Studies**Anticancer**

The effect of crude polysaccharide isolated from *S.nigrum* linn. (SNL-P) was examined both in vivo and in vitro on U14 cervical cancer cells. Though exposure to SNL-P had not antiproliferative effect in vitro at doses up to 1 mg/ml, it decreased the number of ascites tumor cells and survival time of U14 cervical cancer bearing mice which received between 90 – 360 mg / kg bw. P.o. FACScan flow cytometer analysis showed that most of the ascites tumor cells were arrested in G2/M phase of cell cycle. This can be considered as the basis for its use as an anticancer agent (Jian et al., 2009). Similarly, in an earlier work by Jian ET. al. (2007) on the in vivo effect of a 12 day oral administration of SNL-P, Showed a significant growth inhibition effect on cervical cancer (U14) of tumor bearing mice with increased expression of Bax and a decreased expression of Bcl-2 and mutant p53 which had a positive correlation with the number of apoptosisign tumor cells. Moreover, SNL-P treatment decreased the level of blood serum TNF α , this corresponds to triggering of apoptosis in tumor cells. These finding demonstrated that the SNL-P is a potential antitumor agent (jian et al., 2007). The review by an Lei et al. (2006) suggests that the anticancer potential of Sn was based on its capacity to interfere with the structure and function of tumor cell membrane, disturb the synthesis of DNA and RNA, change the cell cycle distribution, blocking the anti-apoptotic pathway of NF KappaB, activating caspase cascades reaction and increasing the production of nitric oxide. The contribution of autophagic cell death in the anticancer pathways of Sn was carefully elucidated through studies utilizing LC3-I and LC3-II proteins in Hep G2 Cells. Results show a concentration dependent mechanism of sn in cell autophagy and vacuolization. This may provide a leverage to treat liver specific cancer.

Immunomodulatory Effects

In vivo experience showed that the ratio of CD4 $^{+}$ /CD8 $^{+}$ peripheral blood T-lymphocyte subpopulations were restored following the treatment of SNL-P. Furthermore, treatment with SNLP also caused a significant increases in INF- γ (p<0.01, 90, 180 and 360 mg/kg bw) and a remarkable decrease in IL- β (p<0.01, 90, 180 mg/kg b.w.; p<0.05, 360 mg/kg b.w.) measured by the method of ELISA. These data showed that SNL-P possess potent antitumor activity and SNL-P possess potent antitumor activity and SNL-P might exert antitumor activity via activation of different immune responses in the host rather than by directly attacking cancer cell on the U14 cervical cancer bearing mice. Thus, SNL-P could be used as an immunomodulator (Jian et al., 2009).

Hepatoprotective Effects

Study by Hsu et al. (2009) utilized 2 – acetylaminofloreneas an inducer of hepatocarcinogenesis which is consistent with increased expression of glutathione S-transferase-alpha and – mu, the level of transcription factor Nrf2, glutathione peroxides, superoxide dismutase – 1, and catalyses (Hsu et al., 2009).

Hypolideamic, Anti-Hyperglycemic and Hypotensive Potentials

Hyperlipidemia is a major risk factor in cardiovascular dysfunction is promoted by excessive agitation of the cation pumps on the cell membranes. It is therefore, conceivable that since Sn had inhibitory effects on the H $^{+}$ K $^{+}$ ATPase, it could serve as cardio protective regimen. Hypolipidemic agents are the first defense against lipid associated pathologies, therefore, the investigation of the effects of 150 kDa glycoprotein isolated from *S. Nigrum* Linn. (SNL), which has been used as a hepatoprotective and anticancer agent in folk medicine, is necessary. Mice treated with Sn had decreased levels of the plasma lipoprotein levels (TG, TC and LDL). In addition, SNL glycoprotein inhibits them activity of cholestyramine induced hepatic HMG – CoA reductase at 40 g/g head body weight (lee et al., 2005). Validation of the ethno botanical use of the leaves of *S.nirgum* Linn. (Solanaceae), *Vitex negundo* Linn. (Verbenaceae) and stems of *Nopalea Cochinelifera* (Linn.) as anti – diabetic agents using the oral glucosetolerance test showed that there was no significant lowering in BGLs by *S.nirgum* (villasenor and Lamadrid, 2006).

Antioxidant

A 2006 study of commonly consumed roots crops in the Philippines – Kamote (*Ipomoea batata*); ubi, purple yam (*Dioscorea alata*); cassava (*Manihot Esculenta*); taro or gabi (*Colocasia esculenta*); carrot (*Daucus Carota*); yacon (*Smallanthus sonchifoliosus*) showed them to be rich sources of phenolic compounds with antioxidant activity, highest in sweet potato, followed by taro, potato, purple yam and lowest in the carrot.

- Anticonvulsant: A study showed potato juice exerted significant anticonvulsant activity in mice. It suggests that potato juice, as well as potato, may influence brain GABA activity.
- Teratological and Toxicological Studies : study was done on the effects of acute and chronic administration in pregnant and non pregnant rats of alkaloidal, glycoalkaloidal and phenol compounds from *Solanum tuberosum*. None of the compounds produced neural tube defects; a few fetuses had rib abnormalities.
- Antiobesity of New Purple Potato Variety: Study of a purple potato variety showed antiobesity potential via inhibition of lipid metabolism through p38 MAPK and UCP – 3 Pathways.

Toxicity**Potato Poisoning**

- Occurs when someone eats the green tubers or new sprouts of the potato plant. The poisonous ingredient is Solanine which is very toxic even in small amounts. Potatoes should never be eaten when spoiled or green below the skin. Sprouts should always be discarded.
- Symptoms: Delirium, diarrhea, dilated pupils, fever or hypothermia, hallucinations, headache, numbness, paralysis, shock, vision changes, vomiting.

- Treatment: do not attempt home treatment or alternative or alternative remedies. Seek immediate medical help. Depending on severity, treatment might necessitate use of activated charcoal, breathing support, IIV fluids and gastric lavage.

Availability - Wild – Crafted use of leaves Cultivated for the potato [3]

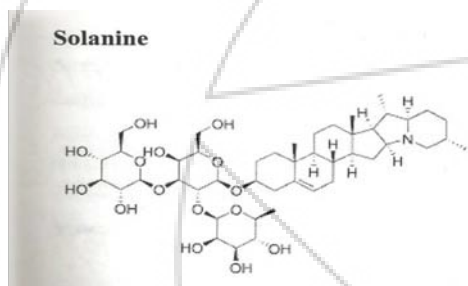
Glycoalkaloid

Some steroidal alkaloid contains a sugar molecule. They are referred as alkaoidal glycosides.

E.g. Solanum contain a complex alkaloidal glycoside solanine.

Glycoalkaloids principally solanine and chaconine are present at variable concentration in solanum species. Glycoalkaloids are synthesized in leaves and then translocated to the different plant organs.

Solanine



Solanine is an alkaloid toxin found in members of the nightshade family, such as eggplants, potatoes, tomatoes, and the infamous deadly nightshade or belladonna. This toxin is part of the plant's defense mechanism, and it is designed to make nightshades unappealing and deadly to animals which might attempt to eat them. Most animals, including humans, have learned the lesson and learned to leave nightshades alone. Leaves, roots, flowers, stems, and fruits can all contain solanine in varying levels.

This toxin has neurological and gastrointestinal effects. When it is ingested in large enough quantities, it can cause nausea, cramps, vomiting and diarrhea, in addition to symptoms like confusion, dizziness, difficulty walking, and slurred speech. Eventually, the body will become overloaded with the toxin, causing organs to fail and eventually leading to death or severe injury. Solanine appears to affect the mitochondria of the cells as it spreads through the body.

Humans eat many members of the nightshade family, although some societies were initially suspicious of foods like potatoes and tomatoes when they were imported from the New World because of concerns about known toxins. Usually, the solanine levels in things like eggplant, potatoes, and tomatoes are too low to cause health problems. However, there are circumstances in which solanine can be elevated. Unripe tomatoes tend to have higher levels of the toxin, as to potatoes which have been

damaged or exposed to the sun, because the plants from more solanine in response to perceived threats. Sprouts of potatoes and tomatoes also have high levels of the toxin.

The toxin is heat – stable, but it will eventually break down at high temperatures. Deep frying temperatures of over 1700F (about 760C), for example, can reduce the risk of solanine toxicity, but baking or microwaving is not as effective, and boiling won't work because the toxin will leach into the water. People who are concerned about the toxin can avoid unripe tomatoes and potatoes which have started to turn green, as the green color indicates that the potato has been exposed to the sun. While the green color itself is harmless, it shows that the potato has been able to photosynthesize, which requires sun exposure.

Historically, solanine was used in the treatment of epilepsy and asthma, in controlled doses. This practice is no longer common, as there are safer and more effective ways to treat these conditions. Solanine also has fungicidal and pesticidal qualities, but extraction and processing of this toxin is so time consuming that the substance is rarely used for these purposes. Another compound found in nightshades is atropine, another alkaloid toxin which is widely used in controlled amounts for various medical applications.

Solanine Poisoning

Symptoms

Solanine poisoning is primarily displayed by gastrointestinal and neurological disorders. Symptoms include nausea, diarrhea, vomiting stomach cramps, burning of the throat, cardiac dysrhythmia, headache and dizziness. In more severe cases, hallucinations, loss of sensation, paralysis, fever, jaundice, dilated pupils, Hypothermia and death have been reported. In large quantities, solanine poisoning can cause death. One study suggests that doses of 2 to 5 mg per kilogram of body weight can cause toxic symptoms, and doses of 3 to 6 mg per kilogram of body weight can be fatal. Symptoms usually occur 8 to 12 hours after ingestion, but may occur as rapidly as 30 minutes after eating high solanine foods. The lowest dose to cause symptoms of nausea is about 25 mg solanine for adults, a life threatening dose for a regular – weight adult ranges about 400 mg solanine.

One Study Suggests the Toxic Mechanism

Mechanism of action of solanine is caused by the chemical's interaction with mitochondrial membranes. Experiments show that solanine exposure opens the potassium channels of mitochondria, decreasing their membrane potential. This in turn leads to Ca²⁺ being transported from the mitochondria into the cytoplasm, and it is this increased concentration of Ca²⁺ in the cytoplasm that triggers cell damage and apoptosis.

Correlation with Birth Defects

Some studies show a correlation between the consumption of potatoes suffering from late blight (which increases

solanine and other glycoalkaloid levels) and the incidence of congenital spina bifida in humans. However, other studies have sworn no correlation between potato consumption and the incidence of birth defects.

Plan of work

- Selection of topic
- Collection of leaves.
- Drying of leaves
- Isolation of solanine from potato leaves.
- Evaluation of solanine by chemical test.
- Thin layer chromatography
- IR
- Interpretation of data.
- Antimicrobial Activity.
- Compilation of data.

The Objectives of present study are;

1. To explore the possibility of using traditional medicinal plant with proper chemical and pharmacological profile.
2. Isolation of solanine from leaves of potato plant.
3. To evaluate the antimicrobial activity of solanine.

Experimental Work and Results

Plant Material and Isolation

The leaves of *Solanum tuberosum* was collected from tropical and subtropical region and was authenticated by Department of Botany, Y.C. Institute of Science, Satara. The dried leaves are used for the activity.

The leaves of plant were shade dried, finely powered and subjected to isolation following the method of Siddiqui book of chemistry.

1. 100 gms of finely powered potato leaves are macerated with 5% acetic acid (300ml) in a beaker for 24 hours.
2. Boiled of this material upto 700c and cooled.
3. After cooling, pH of solution was adjusted upto 9.5 with ammonia and again cooled.
4. Centrifuged, washing the precipitate with sufficient amount of 1% ammonia solution and again centrifuged.
5. Discarded the supernant liquid and washing.
6. Residue was dried and weighed.
7. In this way solanine was isolated.
8. This isolated solanine were resuspended in respective solvents to get 100ug/ml for antimicrobial activity.[5]

Thin Layer Chromatography

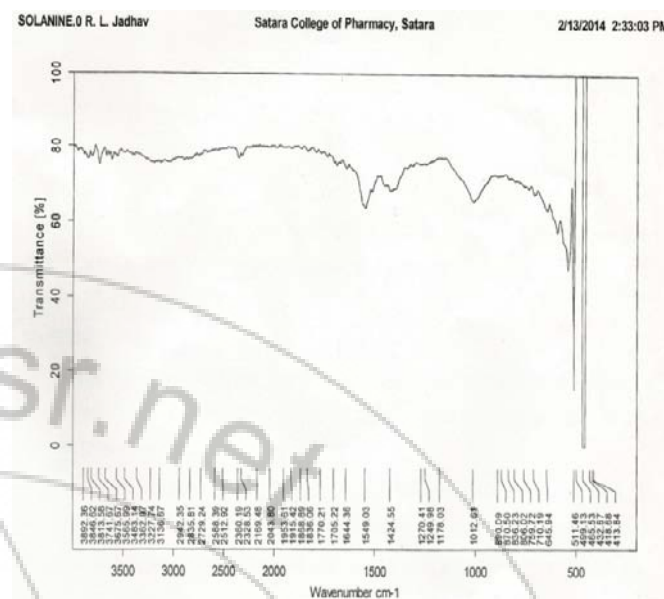
Chromatography was performed by using Chloroform: Methanol: Ammonia (7:3:0:5) solvent system for the analysis of glycoalkaloid solanine.

Detection

The developed chromatograms were observed under U.V.254nm.

Compound	Rf Value
Solanine	0.45

IR Graph of Solanine



- 1) The presence of peak at 3741.67 in the graph shows the structure – O-H is present in the sample.
- 2) The presence of peak at 1549.03 in the graph shows the structure – C=C is present in the sample.
- 3) The presence of peak at 1012.61 in the graph shows the structure – C-O lactone bonding is present in the sample.
- 4) The presence of peak at 1424.55 in the graph shows the structure –C-H- bending cycloalkane is present in the sample.
- 5) The presence of peak at 1249.98 and 1178.03 in the graph shows the structure weak –C-N- stretching is present in the sample.

Antimicrobial Activity

Gram Positive Bacteria – *Bacillus subtilis* and *Staphylococcus aureus*.

Preparation of Sub – Culture

- One day prior to these testing, inoculations of above bacterial cultures were made in the nutrient agar.
- Preparation of base layer medium (nutrient agar)
- It was prepared by dissolving definite volumes of nutrient agar 1.68gm, in 30ml of distilled water and sterilized by autoclaving at 15 p.s.i. for 20 minutes.
- Sterilization of equipments:
- Petri dishes, pipettes, cork borer, test tubes were sterilized by dry heat sterilization at 1600c for 1 hr in hot air oven.
- Preparation of test solution:
- Isolated solanine (100 mg) were dissolved in acetone to give a 100 g/ml of these stock solution was pipette out with help of micropipette and used for testing.
- Method of testing:
- Firstly we prepared sterilized nutrient agar medium and poured into the petridish (25ml). After that near about (0.1ml) bacterial inoculum were added (*Bacillus subtilis* and *E.Coil*) into the different two petridishes respectively.

- Immediately the cups were prepared with help of cork borer (2 cups). In each cups then we added (0.1ml) of test and standard drug solution. Finally it was kept for diffusion in a deep freezer for 2 hrs. It was removed and placed in cubator at 370 c. for 48 hrs. After incubation, the zone of inhibition was measures in mm and is reported. [6-8]
- Ciprofloxacin was used as a standard drug.

Discussion and Conclusion

The Potato plant leaves were macerated with 5% acetic acid in a breaker for 24 hrs. This macerated material boiled up to 700c and cooled. After cooling, pH of solution was adjusted up to 9.5 with ammonia solution. Centrifuged, washing the precipitate with 1% ammonia solution and again centrifuged. Discarded the supernant liquid and washing. Residue was dried and weighted. In this was solanine was isolated.

The isolated solanines were evaluated by using following chemical tests:

Test	Observation	Inference
Test Solanine + Sulphuric acid + formaldehyde	Violet red colour observed	Solanine present
Test Solanine + Libermann burchard reagent	Cherry red colour observed	Solanine Present

The isolated solanine were analyzed by using following:

1) TLC showed following results,

The developed chromatograms were observed under U.V. 254 nm

Compound	Rf Value
Solanine	0.45

2) IR analysis

Functional Group	Observed Range	Standard Range
Phenol (-O-H Str.)	3741.67	3100-3700
Aromatic (C=C Str.)	1549.03	1450-1600
Cycloalkane (-C-H)	1424.55	1270-1450

Above results indicates that solanine were separated successfully.

Antimicrobial activity of solanine against Bacillus subtilis:

Glycoalkaloid	Test	Standard
Solanine	5 mm	12 mm

Antimicrobial activity of solanine against Staphylococcus aureus:

Glycoalkaloid	Test	Standard
Solanine	3 mm	12 mm

The antibacterial activities of solanine were examined against Bacillus subtilis. Antimicrobial activity was investigated by cup plate method. The solanine showed effective inhibition against the Bacillus subtilis and also solanine showed slite inhibition against the staphylococcus aureus. Therefore the leaves of potato can be considered to be the promising source of antimicrobial compounds.

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