EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF SOLANUM AMERICANUM MILL ETHANOLIC AND AQUEOUS LEAF EXTRACTS

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ABSTRACT

OBJECTIVE: Solanum Americanum mill is paramount in medicinal perspective and belong to family Solanaceae from so many different parts of the plants, significant pharmacological and biological activities have been reported. Previously, Solanum Americanum mill. Leaves extract contains biologically active ingredients such as Alkaloids, Flavonoids, Steroidal Glycosides, Saponins, Tannins, Triterpenoids, Glycosides, which possess a wide range of pharmacological properties, Solanum Americanum mill extract was found to contain appreciable amounts of vitamins & minerals as well, this study was aimed to analyze the presence of various phytoconstituents and to determine the Anti-artheritic potential In Vitro.

RESULT: The finding of this study is the % inhibition by Bovine serum albumin Protein denaturation method and Egg albumin denaturation method, by using both ethanolic and aqueous extracts in various concentration (100%, 200%, 400%, 800%, 1000%) and comparing the obtained result with the standard (Diclofenac Sodium) which is using as Standard.

CONCLUSION: The present finding suggest that the Solanum Americanum mill contains various phytoconstituents & it also possess significant In vitro Anti-artheritic activity, which is more than the standard drug (Diclofenac Sodium).

Keywords: Solanum Americanum mill, Anti-artheritic, Protein denaturation, % inhibition.

INTRODUCTION: Arthritis is the chronic inflammatory disease that affect the various Parts of the joints include synovium of tendons cartilage and the muscles, Joints can be inflamed for different types of reasons and that result in cartilage damage, swelling and limited movement. Erosion of the underlying bone is the characteristic of the chronic arthritis. Approximately 1% of the world-wide Population affects by this Rheumatoid arthritis Problem. The etiology us not known yet. This is called as the inflammation of the synovial joint due to the disturbance of immune mediate response. It has been reported that microorganisms including different variety of bacteria, viruses, fungi, and parasites, and also Bacteria toxin and Bacterial DNA may increase the inflammatory response at the bone and joint. According to population studies 10-20% of all people of 65 years or older than that either are currently receiving a Prescription for the Non-steroidal anti rheumatic drugs. and now the number increase from. 380 million to 600 million is recorded. More than the 30 million people worldwide take the gliding drugs, aver time, and the 40% of the purchaser are older than 60 years.
Not all the anti-inflammatory drugs suppress the T-cell and the b-cell that’s why not all the Anti-inflammatory drugs characterize as the anti-arthritic drug. The Availability of thousands of medicinal plants in different bioclimatic condition makes India “Emporium of medicinal plants”. A big range number of medicinal plants have been tested and found to continue active constituents with the curative properties against the arthritic disease. Herbal drugs enhance the resistance against infections on human body thus, increase the human immunity. Plants are the source of natural chemicals thus the use of the traditional medicines are widely spread and the chemical which taken out from the plant might serve as leads on the development of the drugs. Herbal medicines are popular due to their non/less toxicity and side effects in comparison of the allopathic medicines. A large number of medicinal plants is dated back 3500 B.C. The curative of plant has been mentioned in the suktas of rived and Atharvaveda. Ayurveda. The ancient well-known treatise in Ayurveda, the charka Samhita were written by charka and surut respectively. World health organization estimated that 80% of the world’s inhabitants still rely mainly on traditional medicines for their health care [1-3].

Medicine from plant sources have been in use in Homeopathy, Ayurvedic, Allopathy and also in traditional medicines since time immemorial. A medicinal plant plays a significant role among the modern and traditional systems. Their use has been multiplied through various researches and application due to a number of side effects from use of synthetic drugs, antibiotics and high cost. The people of rural area mainly depending on the traditional medicine for curing their ailments cause of the non availability of modern medicines and hospitals. In India, with more than 75% of the population residing in rural areas [4] close to the natural resources, rich traditions of utilizing medicinal plants have existed among indigenous people of ages [5-9].

USES OF HERBAL DRUGS IN MEDICINES:

In India, Herbal drugs are important part of Indian Medicine system (Ayurveda) which is an ancient and conventional system. Moreover, this our culture is rich in herbal drugs in that way causing a high incidence of their self-medication, as also these drugs are sold openly [10].

HISTORY OF ARTHRITIS:

Arthritis has an impact on people of all ages and it has been known to mankind since ancient times, little was known of the diseases, Except its symptoms and signs. For example, Rheumatoid Arthritis can be traced back to dinosaurs and prehistoric man. According to the Book “The complete Dinosaur” only a small portion of Dinosaurs actually suffered anything resembling human Arthritis, on another hand, Fossil records show evidence that other forms of Arthritis did affect dinosaurs, Specifically Gout, A deep detailed examination by Rothschild of the Bones of a Tyrannosaurus Rex showed the distinctive holes found in the bones of Gout Patients [11-14].

HERBAL THERAPY FOR THE TREATMENT OF ARTHRITIS:

Herbal medicines are used in the treatment of various ailments from ancient times and it is not an exaggeration to say that the use of the herbal drugs is as old as mankind. Herbal medicines are synthesized from the therapeutic experience of generation of practicing physicians of the ancient system of medicine for more of than hundreds of years [15]. Nowadays, researcher shows great interest in those medicinal agents that are derived from plants because the currently available drugs are either have certain side effects or are they highly expensive. Nature has
blessed us with enormous wealth of herbal plants which are widely distributed all over the world as a source of therapeutic agents for the prevention and cure of various diseases. According to WHO, world’s 80% population uses herbal medicines for their primary health care needs. Herbal medicines will act as parcels of human society to combat disease from the dawn of civilization [16,17]. The medicinally important parts of these herbal plants are chemical constituents that produce a desired physiological action on the body Since ancient time India uses herbal medicines in the officially alternative systems of health such as Ayurveda, Unani, Siddha, Homeopathy, and Naturopathy. In India, there are more than 2500 plants species which are currently used as herbal medicaments. For than 3000 years, the herbal medicines are used either directly as folk medication or indirectly in the preparation of recent pharmaceuticals. Thus, from the knowledge of traditional plants, one might be able to discover of new effective and cheaper drugs. In this review article, we have tried to cover all the ayurvedic strategies that are followed for the treatment of RA without any possible side effects. The future treatment of RA should provide more effective relief [18-21].

GENERAL CONSIDERATION OF ARTHRITIS RA Can Be Classified As: [22-28]

- Palindromic rheumatoid arthritis
- Juvenile rheumatoid arthritis
- Rheumatoid spondylitis
- Other types of arthritis
- Osteoarthritis There are of two types of osteoarthritis –
  a) Primary osteoarthritis - It occurs in the elderly.
  b) Secondary osteoarthritis- It occurs at any stage of life.
- Ankylosing spondylarthritis
- Infectious arthritis
- It can be classified as fallows
  a) Supportive arthritis
  b) Tuberculous arthritis
  c) Lyme arthritis
  d) Viral arthritis
- Gout and Gout arthritis

TYPES OF ARTHRITIS: [29]

There are over 100 types of arthritis. Here is a description of some of primary common ones.

OSTEOARTHRITIS(OA): [30,31]

Synonymously it is called ‘Azhal keel Vayu’ in siddha system of medicine, it is the most common form of joint disease, and it is characterized by erosion of articular cartilage.
This joints mostly affected by OA are the knees, spine, and hands, although others joint also may be involved. It is classified as

1 –Primary (Idiopathic)

2-Secondary

➢ RHEUMATOID ARTHRITIS (RA): [32,33]

Synonymously called ‘Vali Azhal keel Vayu’ in siddha system of medicine, it is a chronic disease, usually manifested as inflammation of multiple joints ,and the severity of this disease is varies from person to person.

➢ INFECTIOUS ARTHRITIS:

➢ Neisseria gonorrhoea, and other bacteria are the most common causes of infectious arthritis, synonymously called as ‘lyak keel Vayu’ in siddha system. Septic or infectious, arthritis is infection of one or more joints by microorganisms. Normally, the joint is lubricated with a small amount of fluid that is referred to as synovial fluid or also as joint fluid. The normal joint fluid is sterile and if removed and cultured in the laboratory, no microbial growth will be found. With septic arthritis, microbes are identifiable in an affected joint fluid [34,35].

➢ JUVENILE RHEUMATOID ARTHRITIS (JRA):

Juvenile rheumatoid arthritis is a chronic, inflammatory autoimmune joint disease. It is the common rheumatic disease in children and adolescents. It is defined as ‘persistent arthritis of unknown etiology that begins before the age of lars and persists for at least 6 weeks.’ It is diagnosed after excluding other infections may trigger the condition in genetically susceptible children. However it is an unusual for more than one child in a family to have arthritis [36-38].

Pathogenesis of RA

Wbc Of Immune System Moves into Joints

Release Cytokine Which Attacks the Cell of Synovial Membrane

Release other destructive substance

Synovial membrane grows new blood vessels to form a pannus.

Pannus grows and destroy area of cartilage and bones inside the joint.

Inflammation causes fluid buildup in the joint making joint swell.

Joint space narrow.
MATERIALS AND METHODS

Materials: All reagents procured were analytical grade.

PROCEDURE:
Collection of plant
Collection of Solanum Americanum Mill leaves are collected from local areas of Dehradun (Uttarakhand).

Drying and size reduction
Collection of Solanum Americanum Mill leaves from local areas and shade dry under the room temperature without the sun exposure for 6 days. The dried leaves further crushed to powder and ready for extraction process.

Preparation of ethanolic extract
Process- Take 20 gm of air-dried drug weight accurately by using weighing balance and macerated with 200 ml ethanol in closed conical flask for 48 hr.
It was frequently shaken during the 6 hr. and allowed to stand for 1 day filtered it out.
Heated the filtrate for 24 hr. at 50°C and pour in Petri-dish, dry it for 2 to 3 days and dried powdered was collected from Petri-dish.

Preparation of aqueous extract
Process-
- Dried leaf powder of Solanum Americanum Mill macerated with chloroform water.
- The mixture stirred frequently at an interval of 3 hr.
- After 3 days solvent replaced with fresh solvent and maceration perform as mention above for 2 times.
- The filtrate filtered through muslin cloth followed by Whatman no.1 filter paper.
- The extract of Solanum Americanum Mill obtain store in vacuum desiccators after freeze drying.

Preparation of buffer solution

Process- Phosphate buffer solution:
Phosphate buffer saline (6.4)
1. Disodium hydrogen phosphate =1.18 gm
2. Potassium dihydrogen phosphate =0.9 gm
3. Sodium chloride=8.00 gm
4. Water = 500 ml

Preparation: Dissolved 2.5 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate, 8.2 gm of sodium chloride in 500 ml of distilled water. Adjust pH of the solution to 6.3 with 1 M sodium hydroxide of 1 M hydrochloric acid.

Preparation of Egg albumin
Using a glass rod break the outer shell of the egg.

The colourless liquid and yellow yolk is collected in porcelain dish

100 mL 5% Nacl solution is prepared and added in 250 mL volume beaker

Pipette out albumin and pour in beaker containing Nacl solution with constant stirring

Stir it for around 15 to 20 minutes

With the help of filter paper and funnel, filter the contents of the beaker

Label the filtrate as, Egg albumin solution.

**In vitro Method used for anti-arthritic activity using Ethanolic extract.**

1) BOVINE SERUM ALBUMIN (BSA) DENATURATION METHOD

The following 4 solutions were used.

**Test solution** (0.5ml) consist of 0.45ml of BSA (5%w/v) and 0.5 ml of extracts of Solanum Americanum Mill in 100%, 200%, 400%, 800%, 1000%, concentration.

**Test control solution** (0.5ml) consist of 0.45ml of BSA (5%w/v) and 0.05ml of distilled water

**Product control** (0.5ml) consist of 0.45 ml of distilled water and 0.05ml of extracts of Solanum Americanum Mill in 100%, 200%, 400%, 800%, 1000%, concentrations.

**Standard solution** (0.5ml) consist of 0.45 ml of BSA (5%w/v) and 0.05 ml of diclofenac sodium in 100%, 200%, 400%, 800%, 1000%, concentrations.

The pH of the above solutions was adjusted to 6.3 by using small amount of 1 N HCl, the samples were incubated at 37°C for 20 min and heated at 57°C for 5min which was cooled and 2.5 ml of phosphate buffer (ph6.3) was added to it UV- Visible Spectrophotometer (Analab) was used to measure the absorbance at 435, 340, 435, and 325 nm for the following solutions –test solution, standard solution, product control, and test control solution. The control represents 100% protein denaturation and the result were compared with standard diclofenac sodium.
The percentage inhibition of protein denaturation was calculated by using the formula (Mizushima et.al 1968)

\[
\text{Percent inhibition} = 100 - \frac{\text{optical density of test solution} - \text{optical density of (product control*100)}}{\text{optical density of test control}}
\]

II) INHIBITION OF ALBUMIN DENATURATION (EGG ALBUMIN)

**Methodology**

The following three solutions were used.

**Test solution**

5 ml of test solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and 2 ml of in various concentrations of extracts (100%, 200%, 400%, 800%, 1000%, µg/ml).

**Test control solution**

5 ml of test control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffered saline and 2 ml of distilled water.

**Standard solution**

5 ml of standard solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and diclofenac sodium in (100%, 200%, 400%, 800%, 1000%, µg/ml concentrations).

The pH of the above solutions was adjusted to 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 70°C for 5 min denaturation’s, and the results were compared with standard diclofenac sodium.

After cooling, their absorbance was measured at 660 nm using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for the determination of absorbance. (49)

The percentage inhibition of protein denaturation was calculated as follows:

\[
\text{Percentage inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100
\]

**4.5 EXPERIMENTAL AND RESULTS**
Figure No. 1.0. Evaluation of Anti Arthritics Activity
Table no.1- In vitro anti-arthritic activity of leaves of Solanum Americanum Mill by Bovine serum albumin (BSA) denaturation method using aqueous extract.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TYPE OF EXTRACT</th>
<th>CONCENTRATION (µg/ml)</th>
<th>%INHIBITION OF DENATURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AQUEOUS</td>
<td>100 (µg/ml)</td>
<td>13.0</td>
</tr>
<tr>
<td>2</td>
<td>AQUEOUS</td>
<td>200 (µg/ml)</td>
<td>30.5</td>
</tr>
<tr>
<td>3</td>
<td>AQUEOUS</td>
<td>400 (µg/ml)</td>
<td>57.16</td>
</tr>
<tr>
<td>4</td>
<td>AQUEOUS</td>
<td>800 (µg/ml)</td>
<td>70.04</td>
</tr>
<tr>
<td>5</td>
<td>AQUEOUS</td>
<td>1000 (µg/ml)</td>
<td>84.24</td>
</tr>
<tr>
<td>6</td>
<td>DICLOFENAC SODIUM</td>
<td>100 (µg/ml)</td>
<td>56.03</td>
</tr>
<tr>
<td>7</td>
<td>DICLOFENAC SODIUM</td>
<td>200 (µg/ml)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>DICLOFENAC SODIUM</td>
<td>400 (µg/ml)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>DICLOFENAC SODIUM</td>
<td>800 (µg/ml)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>DICLOFENAC SODIUM</td>
<td>1000 (µg/ml)</td>
<td></td>
</tr>
</tbody>
</table>

Table no.2- In vitro anti-arthritic activity of leaves of Solanum Americanum Mill By Bovine serum albumin (BSA) denaturation method using ethanolic extract.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TYPE OF EXTRACT</th>
<th>CONCENTRATION (µg/ml)</th>
<th>%INHIBITION OF DENATURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ETHANOLIC</td>
<td>100 (µg/ml)</td>
<td>27.54</td>
</tr>
<tr>
<td>2</td>
<td>ETHANOLIC</td>
<td>200 (µg/ml)</td>
<td>41.88</td>
</tr>
<tr>
<td>3</td>
<td>ETHANOLIC</td>
<td>400 (µg/ml)</td>
<td>80.38</td>
</tr>
<tr>
<td>4</td>
<td>ETHANOLIC</td>
<td>800 (µg/ml)</td>
<td>96.28</td>
</tr>
<tr>
<td>5</td>
<td>ETHANOLIC</td>
<td>1000 (µg/ml)</td>
<td>99.28</td>
</tr>
<tr>
<td>6</td>
<td>DICLOFENAC SODIUM</td>
<td>100 (µg/ml)</td>
<td>56.03</td>
</tr>
<tr>
<td>7</td>
<td>DICLOFENAC SODIUM</td>
<td>200 (µg/ml)</td>
<td>106.6</td>
</tr>
<tr>
<td>8</td>
<td>DICLOFENAC SODIUM</td>
<td>400 (µg/ml)</td>
<td>159.19</td>
</tr>
<tr>
<td>9</td>
<td>DICLOFENAC SODIUM</td>
<td>800 (µg/ml)</td>
<td>287.88</td>
</tr>
<tr>
<td>10</td>
<td>DICLOFENAC SODIUM</td>
<td>1000 (µg/ml)</td>
<td>290.6</td>
</tr>
</tbody>
</table>
RESULT:

% inhibition of denaturation of protein by various ethanolic and aqueous extracts with different concentration of 100 (µg/ml), 200 (µg/ml), 400 (µg/ml), 800 (µg/ml), 1000 (µg/ml), were
performed and result obtained as 27.54%, 41.88%, 80.38%, 96.28%, 99.28%, with ethanolic extract, and 13.0%, 30.5%, 57.16%, 70.04%, 84.24%, with aqueous extract, by using Bovine serum albumin (BSA) denaturation method in which extract with the concentration of 1000(µg/ml) have higher % inhibition (99.28% with ethanolic extract) and (84.24% with aqueous extract), similarly, % inhibition of denaturation of protein by various ethanolic and aqueous extracts with different concentration of 100 (µg/ml), 200 (µg/ml), 400 (µg/ml), 800 (µg/ml), 1000 (µg/ml), were performed and result obtained as 50.2%, 101.6%, 186.7%, 260.07%, 271.08%, with ethanolic extract, and 37.8%, 68.4%, 172.17%, 220.02%, 228.05%, with aqueous extract, by using albumin denaturation (egg albumin), in which extract with the concentration of 1000(µg/ml) have higher % inhibition (271.08% with ethanolic extract) and (228.05% with aqueous extract), thus it is more potent as an anti-arthritic herbal drug. While % inhibition by standard drug with different concentration of 100 (µg/ml), 200 (µg/ml), 400 (µg/ml), 800 (µg/ml), 1000 (µg/ml), were performed and result obtained as 56.03%, 106.6%, 159.19%, 287.88%, 290.6%, is less (56.03%) then test.

DISCUSSION:

Denaturation of tissue protein is one of the known causes of arthritic disease, so the substance which have the ability to prevent protein denaturation could be used as anti-arthritis drug. Various extracts of leaves of Solanum Americanum Mill were screened for the anti-arthritic activity using protein denaturation method & Egg albumin denaturation in which the various extracts of Solanum Americanum Mill showed anti-arthritic potential in a dose dependent manner when compared to that of diclofenac sodium [39, 40].

In the present study of protein denaturation, the increments in absorbances of test samples with respect to control indicated stabilization of protein (albumin) denaturation by Solanum Americanum Mill and reference drug diclofenac sodium [41]. Solanum Americanum Mill contain alkaloids, flavonoids, tannins are known to promote antiarthritic activity.

Summary & Conclusion:

In the view of above discussion, it is conceivable that Solanum Americanum mill has been observed to exert significant Anti-arthritic effect in experimental studies. The ethanolic extracts of Solanum Americanum mill showed some better Anti-arthritic activity then the aqueous extracts. Result attributed the presence of active principle such as active glycoalkaloid, steroidal alkaloids, tannins, steroidal saponins, steroid alkaloid and glycoprotein which are responsible for this Anti-arthritic activity Hence, it could be beneficial for further work as anti-arthritic agent.

Hence, the results of this study reveal the extracts of Solanum Americanum mill were capable of controlling the production of autoantigens and inhibit denaturation of protein especially denaturation of albumin.

The present studies indicates that extracts of Solanum Americanum mill exhibit strong anti-arthritic property which could be a potential source of anti-arthritic property.

The inhibition of protein denaturation, albumin denaturation, was studied to establish the mechanism of anti-arthritic activity of Solanum Americanum mill. Therefore, in this In-vitro studies on the leaves extract of Solanum Americanum mill demonstrate the significant anti-arthritic activity. Hence this plant can be used as potent natural anti-arthritic agent.

From the above study, it is concluded that ethanol extract of Solanum Americanum mill have possessing more anti-arthritic property in comparison of aqueous extract.
Due to inhibition of cyclooxygenase (COX) enzyme it gives the positive result in controlling inflammation and helpful in the treatment of Arthritis. Solanum Americanum mill is highly potent anti-inflammatory herbal drug. We are confident that our data provide mechanistic evidence for anti–arthritic appliance of Solanum Americanum mill as a promising candidate for herbal therapeutic agent of Rheumatoid Arthritis.

So, we can say that Solanum Americanum mill is an effective Anti-arthritis agent experimentally and holds prospect in future (Rheumatoid Arthritis) treatment. Development of a suitable formulation with isolated constituents. The future scope of study involves the isolation of Phytoconstituents and mechanism responsible for the anti-arthritic activity.

REFERENCES:


3- Choudhary Majusha,Kumar Vinip, Malhotro Hithesh,Singh Surender, Medicinal Plants with Potential antiarthritic activity , Journal of International Ethopharmacology.ISSUE-2,Vol-4, Apr-Jan-2015. et.al,

4- P Vaijayanthimala, M Sakthipriya B Sangameswaran,IN-vitro anti-arthritic activity of cissus quadrangularis stem extract,Asian journal of pharmaceutical and clinical research ,Vol 12,Issue 2019,et.al,

5- Acharya E, Pokhrel B. Ethno-medicinal plants used by Bantar of Bhaudaha, Morang, Nepal. Our nature 2006;4:9.6-103

6- A.elsadig, E.S.Alia ,A; Amzari and G. James phytochemistry 46(3) 480-494 (19970),

7- Atanu o.f. A review of the pharmacological aspects of solanum americanum mill, Academic Journals ISSN 1538-2273 vol.6, Jan 2011


11- Hassan J.O , Curtiss R; Infect immunology,1994; 2027-2013


13- Kapoor Bhupinder et.al ;Natural products in treatment of rheumatoid arthritis International Journal of green pharmacy July-Sep 2017,

15- Kumar Aditya, In vitro Evaluation of anti-inflammatory activity of leaves of solanum americanum mill, page no. 11-13 2016,
17- K samraj, A review on anti-arthritic herbs in Siddha medicine Introduction, Article-December 2013,
20- Lees P (2003) Pharmacology of drugs used to treat osteoarthritis in veterinary Practice Inflamma pharmacology 11: 385-399,
22- N Kumar Shravan et.al, In vitro Anti-arthritic activity of Leaves of Physalis Angulatalat, IJPIR Journal, Volume -01 ISSUE-03, July-Sep. 2011,
23- Nyeem bin abu mohammad et.al, A review of pharmacological activites and clinical effects International Journal of Applied Research 2017,
24- N. P Sushil kumar et.al Herbs used in the treatment of arthritic- A Review, World Journal of pharmaceutical and Medicinal Research ISSN 2455-3301 July, 2016,
25- Raju K, Anbuganapathi G, Gokulakrishanan V. Effect of dried fruits of solanum americanum mill against CC14- induced hepatic damage in rats; Biol pharma Bull. 2003; 26: 1618-1619
27- Rani Sudha Yerukali, Reddy Jayasankar V, et.al A Review on solanum americanum mill, World Journal of Pharmacy and Pharmaceutical Sciences Volume 6, ISSN 12, 293-303, ISSN 2278-4357,
28- Saleem Mohamad S.T. et.al Solanum americanum mill A review www./researchgate.net, Vol3, ISSUE 6, 342-345, 2009,

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