



INVESTIGATION OF THE PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITIES IN *DATURA METEL*.Linn

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ABSTRACT

Medicinal plants are of great importance to the health of individuals and communities. A large number of plants are claimed to possess the anti-diabetic, anti-fertility, antihyperlipidaemic, anti-inflammatory, anti-cancer, hepatoprotective and immune modulatory activities in the traditional therapeutic systems. It is now believed that nature has given the cure of every disease in one way or another. *Datura* is known as a medicinal plant and plant hallucinogen all over the world. *Datura* has a very special place in Ayurveda since all parts of the plant namely leaves, flowers, seeds, roots, have been used for a wide range of medication such as treatment of leprosy, rabies, insanity, etc. The extract of *Datura*, however, is a potent poison and its indiscriminate use may lead to delirium and acute poisoning that may lead to death. The active constituents in *Datura* include scopolamine, atropine, hyoscyamine, withanolides (lactones) and other tropanes. The ethanolic extract of leaves of *D. metel* was investigated for its phytochemical properties and analysis for its active chemical ingredients. For qualitative and quantitative phytochemical analysis the ethanol extract of *D. metel* acts as a source of therapeutic agent.

KEYWORDS: *Datura metel* chemical constituents, alkaloids, phytochemical screening, ethanol, extract, anti-diabetic.

INTRODUCTION

Datura metel is a shrub-like perennial herb commonly, known as **devil's trumpet** and **metel**. *Datura metel* grows in the wild in all the warmer parts of the world, such as India and is cultivated worldwide for its chemical and ornamental properties. It was first described by Linnaeus in 1753, but no botanically correct illustrations or descriptions were made until after the New World was settled. It is not possible to be sure about its original home. The plant is an annual herb growing up to 3 ft. high. It is slightly furry, with dark violet shoots and oval to broad oval leaves that are often dark violet as well. The pleasantly-scented 6-8 in. flowers are immensely varied, and can be single or double. Colors range from white to cream, yellow, red, and violet. The seed capsule is covered with numerous conical humps and a few spines. It is similar to *D. innoxia*, but *D. metel* has almost glabrous leaves and fruits that are knobby, not spiny. *D. innoxia* is pilose all over and has a spiny fruit. *D. metel* is one of the 50 fundamental herbs used in traditional Chinese medicine, where it is called yáng jīn huā. However, the ingestion of *D. metel* in any form is dangerous and should be treated with extreme caution.

All parts of *Datura* plants contain dangerous levels of tropane alkaloids (highly poisonous) and may be fatal if ingested by humans or other animals, including livestock and pets. In some places, it is prohibited to buy, sell or cultivate *Datura* plants. *Datura metel* may be toxic if ingested in a tiny quantity, symptomatically expressed as flushed skin, headaches, hallucinations, and possibly convulsions or even a coma. The principal toxic elements are tropane alkaloids. Ingesting even a single leaf can lead to severe side effects.

Datura metel is also known as Devil's apple because of its dangerous qualities and the peculiar effects that follows its administration. *Datura* belongs to the classic "witches weeds," along with deadly nightshade, henbane and mandrake. Most parts of the plants contain toxic hallucinogens and *Datura* has a long history of use for causing delirious states and death. It was well known as an essential ingredient of love potions and witches' brews (Preissel and Hans-George, 2002). All *Datura* plants contain atropine alkaloids such as scopolamine, hyoscyamine and atropine, primarily in their seeds and flowers. Because of the presence of these substances, *Datura* has been used for centuries in some cultures as a poison and hallucinogen (Preissel and Hans-George,

2002; Adams *et al.*, 2005). There can be a 5:1 toxin variation across plants and a given plant's toxicity depends on its age, where it is growing and the local weather conditions.

This variation makes *Datura* exceptionally hazardous as a drug. In traditional cultures, a great deal of experience with and detailed knowledge of *Datura* was critical, in order to minimize harm (Preissel and Hans-George, 2002). Many tragic incidents result from modern recreational users ingesting *Datura*. For example, in the 1990s and 2000s, the United States media contained stories of adolescents and young adults dying or becoming seriously ill from intentionally ingesting *Datura* (CDC, 2002; Leinward, 2006). There are also several reports in the medical literature of deaths from *Datura stramonium* and *Datura ferox* intoxication (Michalodimitrakakis and Koutselinis, 1984; Steenkamp *et al.*, 2004). Children are especially vulnerable to atropine poisoning and their prognosis is likely to be fatal (Taha, 1984; Djibo and Bouzou, 2000). In some parts of Europe and India, *Datura* has been a popular poison for suicide and murder. From 1950 to 1965, the State Chemical Laboratories in Agra, India, investigated 2,778 deaths that were caused by ingesting *Datura* (Preissel and Hans-George, 2002). The U.S. Centers for Disease Control and Prevention reported accidental poisoning resulting in hospitalization for a family of six who inadvertently ingested *Datura* used as an ingredient in stew (Bontayan, 2010). In some places, it is prohibited to buy, sell or cultivate *Datura* plants (Preissel and Hans-George, 2002). The most common *Datura* spp. Noxious to the farm animals are *D. stramonium* (atropine alkaloid) and *D. ferox* (scopolamine, 98 to 100% of total alkaloids) mainly present in Europe and South America, respectively (Piva and Piva, 1995). The presence of alkaloids from *Datura* seeds in feeding stuffs may be responsible for chronic and/or subclinical toxic effects; acute poisoning from *Datura* seeds is rare. Pigs are the most sensitive animals to *Datura* poisoning, followed by cattle, horses and chickens (Piva and Piva, 1995). Sheep and rabbits are indifferent to atropine presence in food because they synthesize the atropine esterase enzyme (Piva and Piva, 1995).

The progressive atropine poisoning in pigs leads to a reduction of feed intake and growth, gastrointestinal motility and secretory activity, extreme mouth dryness, increased respiration and

Cardiac rate, pupil dilation, etc. Clinical symptoms are partly similar among different species (Piva and Piva, 1995). Toxic effects from *Datura* alkaloids presence in feeds showed possible variability of alkaloids content tolerated by pigs. It was also reported by Piva and Piva (1995) that, the threshold limit in pigs (20 to 60 kg live weight) was 1.5 mg alkaloids/kg of feed (1.21 mg alkaloids/kg live weight). The aim of this study was to validate the ethnomedicinal use and subsequently the isolation and characterization of the chemical

constituents of *D. metal* leaf which will be added to the potential lists of drugs.

MATERIALS AND METHODS

Plant Material

The leaves of the *D. metal* plant were collected from Kothapally near Alphores College Karimnagar Telangana state located at eastern area in September 2015. Whole Plants were collected from mature plants and identified by comparing with herbarium specimens. The voucher specimen was deposited in the Research Laboratory Department of Chemistry SRR Govt. Arts & Science College Karimnagar. The plant organs were cut into small pieces and shade dried at room temperature (20°C) for two weeks, finely powdered plant materials were stored in airtight polythene bags protected from sunlight until use.

Extraction, Fractionation and Isolation

Shade dried of *D. metal* was soaked in methanol for 5 days. The extract were concentrated using electric water bath at 500°C The solvent extract was concentrated under reduce pressure at 400°C using rotavapor, the extracts obtained were suspended in water and successively partitioned with n-hexane, chloroform, ethyl acetate and methanolic fraction. The chloroform fraction was subjected to column chromatography which led to the isolation of two known compounds β - Sitosterol (1) and stigmasterol (2).

The structure of these compounds was elucidated by comparing their physical and spectra data with already reported data in literature.

Two methods of extraction were used in this study; the first was Precipitation by acetone and the second was extraction by ether.

1. Extraction of *Datura metal* Leaves by Acetone:
2. This method of extraction was carried out as follows:-Five grams of Leaves were ground in a seed mill.
3. The powder was extracted with 100 ml of PBS (prepared by dissolving 8.2 gm Na_2HPO_4 and 8.82 g NaCl in 1000 ml distilled water and kept pH at 7.2 by stirring overnight at 4°C. Then was filtrated and made up to 100 ml with phosphate buffer.
4. The obtained extract was centrifuged at 30 g for 1 hour.
5. The supernatant incubated at 70°C for 15 minutes and then cooled to 4°C, before centrifugation at 10000 g for 15 minutes.
6. The supernatant was cooled in an ice bath to 0°C, followed by a slow addition of 23 ml of acetone at 0°C and left for 10 minutes, before centrifugation at 100g for 10 minutes.
7. After that, 161 ml of acetone was added to the supernatant and then centrifuged.
8. The precipitate of the last centrifugation was dissolved in 75 ml buffer saline.

9. The solution was dialyzed overnight against 5-liter phosphate buffer at 4°C. Afterward, sample for biochemical test was taken while the remain was poured in Petri dish and dried at 37°C.
10. The dried extract was scrubbed off the Petri-dishes, placed in labeled, tightly sealed plastic tubes and stored in deep freeze at -2°C.
11. In order to destruct the seeds, fifteen grams of seeds were ground 15-20 times with diethyl ether in a blender for 15 minutes of each time.
12. The mixture was filtered by Whatman No.1 filterpaper.
13. The resulting precipitate (powder) was extracted by stirring overnight at 4°C with 10 volume of cold PBS (prepared by dissolving 0.82 g Na₂HPO₄ and 11.76 g NaCl in 1000 ml distilled water and kept pH at 7.2).
14. The solution was centrifuged in cooled centrifuge at 400 rpm for 30 min.
15. The volume of supernatant was estimated and fractionated with (NH₄)₂SO₄ ammonium sulfate at 0-40% [the determination of ammonium sulfate weight was occurred out according to the volume of supernatant and depending on the saturation table] at 4°C.
16. The solution was centrifuged in a cold centrifuge at 4000 rpm for 30 minutes.
17. The volume of supernatant was estimated and fractionated with ammonium sulfate at 40-60% at 4°C.
18. After centrifugation by cooled centrifuge, the supernatant was fractionated with ammonium sulfate at 60-100%.
19. The obtained precipitate was re-dissolved in ordinary phosphate buffer and dialyzed against five liters of ordinary PBS at 4°C. Then sample for biochemical test was taken, while the remains poured off in the Petri-dishes for drying at 4°C.
20. The dried extract was scrubbed off the Petri-dishes, placed in labeled, tightly sealed plastic tube and stored in deep freeze at -20°C. Solutions of Biochemical Test Reagents Test for Lectin One drop of the extract was added to one drop of human blood on the slide. Agglutination should appear if lectin was present.

Phytochemical Profiling

TABLE: 1 Phytochemical profiling of crude methanolic extract and various fractions of *D. metal*

Chemical components	n-hexan	chloroform	EtOAc	MeOH	Crude
Alkaloids	-	-	-	-	-
Terpenoids	+	+	+	+	+
Flavonoids	-	+	+	+	+
Anthraquinones	-	-	-	+	+
Tannins	-	-	-	+	+
Phlobatanins	-	-	-	-	-
Saponins	+	+	+	-	-
Glycosides	-	-	-	-	-
Reducing sugars	+	-	+	+	+
Steroids	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Phenol	-	+	+	+	+

The chemical tests were performed on the hexane, chloroform, ethyl acetate and methanolic extracts of *Datura metal* using standard procedure to identify the bioactive secondary metabolite.

Test for alkaloids

Alkaloids salts: the aqueous extract of each organs of the plant (25 ml) was stirred with 15 ml of 10 % HCl on a steam bath for 30 minutes. The mixture was extracted then three times with diethyl ether. 1 ml of the aqueous layer was treated with two drops of Wagner's reagent. Formation of brownish precipitate was regarded as evidence for the presence of salts alkaloids in the extract. Or 0.2gm of each fraction was warmed with 2% H₂SO₄ for 2 min. The reaction mixture was filtered and added a few drops of Dragendorff reagent to each filtrate. Orange red precipitate indicates presence of alkaloids.

Free Alkaloids

10 ml of organic layer (diethyl ether) was evaporated to dryness. The residue was then dissolved in 1.5 ml of HCl 2 % and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract and all results were compared with blanks.

Test for Saponins

2 g of the powdered leaves or fruit was introduced into a beaker containing 100 ml of distilled water; the mixture was boiled in a water bath and filtered. The filtrate was completed then to 100 ml with water. In ten test tubes were introduced the following volumes (1, 2 ... 10 ml) of the mother solution. Then the final volume was readjusted to 10 ml with distilled water. All tubes were vigorously shaken for 15s; formation of froth indicated the presence of saponins.

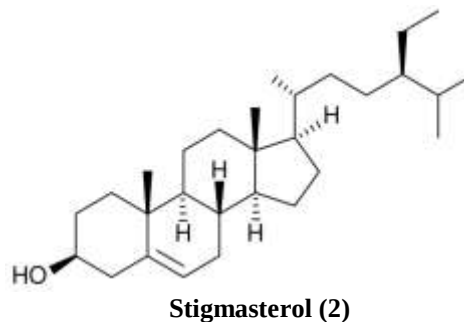
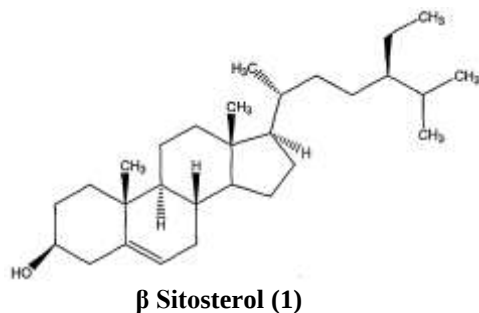
Test for Tannins

A small quantity of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added. A dark green color indicates tannins.

Test for Glycosides

Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Red color indicates presence of Glycosides.

Keywords: present: +, absent: -



Test for Reducing Sugars

Each extract were shaken with distilled water and filtered. The filtrate was boiled with few drops of Fehling solution A and B. An orange red precipitate indicates the presence of sugars

Test for Flavonoids

0.2g of each extract was dissolved in diluted NaOH and few drops of HCl were added. Yellow solutions that turn colorless indicate the presence of flavonoids.

Test for Phlobatanins

0.5 gm of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate indicates the presence of phlobatanins.

Test for Steroids

2ml of acetic anhydride was added to the mixture of 0.5 gm g of each extract and H_2SO_4 (2ml). The color from violet to green in some samples indicates the presence of steroids.

Test for Terpenoids

0.2 gm of each extract was mixed with 2ml of chloroform and concentrated (3ml) H_2SO_4 was carefully added to form a layer. The formation of reddish brown coloration at the interface indicates the presence of terpenoids.

Test for Cardiac glycosides

Keller-Killani test was performed to assess the presence of cardiac glycosides. 5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layed with 1 ml

of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish-blue colour ring may form just gradually throughout thin layer indicated the presence of cardiac glycosides.

Test for Anthracenosides

To 25 ml of methanol extract were added 15 ml of 10 % HCl. The mixture was refluxed for 30 minutes. After cooling, the mixture was extracted three times with 15 ml of diethyl ether extract.

After evaporation of 8 ml of etheric layer, the residue was treated with 2 ml of hot water and some drops of 10 % NH_4OH . Appearance of red oranges colour revealed the presence of anthracenosides.

RESULTS AND DISCUSSION

The results obtained of phytochemical screening of *D. metal* are presented in (Table -1). The n-hexane fraction exhibited the presence of terpenoids, tanins and saponins while rest of hytochemical was not detected. The chloroform fraction showed the accumulation of terpenoids, tanins and saponins, glycosides, reducing sugar and amino acid. When ethyl acetate was tested for various phytochemical tests, it confirmed the presence of terpenoids, tanins and saponins, reducing sugar, beta cyanin and amino acids. The antibacterial effect of various solvent fraction of *D. metal* is presented in (Table-1). The medicinal value of the plant can be correlated to the presence of various bioactive secondary metabolites.





FIG: PHYTOCHEMICAL ANALYSIS AND BIOLOGICAL ACTIVITIES OF *DATURA METAL*. a: Collection of *Datura* plant parts at Kothapally village near Alphores Junior college Karimnagar District b: Air Drying of Plant parts on water absorber filter paper c: Air dried parts of are grinding by Morter and pistil d: Powdered substances dissolved in a solvent and extracted by Rotavapour e: By taking the extract Phytochemical analysis has been doing f: The results of Phytochemical Analysis is kept in the test tubes with stand.

The n-hexane and ethyl acetate fractions and methanolic extract of the *D. metal* showed the presence of terpenoids which exhibits antiviral and antibacterial activities,

Extensive research has been carried out since 1925 for the isolation and characterization of the total alkaloid contents in *Datura* species (Berkov *et al.*, 2006). Vitale *et al.* (1995) has shown that the total alkaloid content in *Datura* varies from 0.02 to 0.52% and scopolamine from 0.0029 to 0.32% relative to the dried material, depending on the geographical area, the part of the plant studied and the stage of growth.

My findings correlate with the observations of previous screened medicinal plants for antimicrobial activity, where most of the active plants showed activity against selected bacterial strains. The pharmacological activity of *D. metal* was confirmed by the antimicrobial assay of various fractions and methanolic extract. Among the tested bacteria *Klebsiella pneumonia* and *Staphylococcus aureus* exhibited complete resistance against all the tested solvent fractions except n- hexane The chloroform

fraction exhibited 16, 12, 10 and 10 mm zone of inhibition against *Klebsiella pneumonia*, *Staphylococcus aureus*, *S. typhimuriun* and *Bacillus subtilis* respectively. While the ethyl acetate was tested against these bacterial strains, it caused activity only against *Staphylococcus aureus* 10 mm zone on inhibition. Methanol fraction demonstrated moderate antibacterial effect against *Klebsiella pneumonia*, *S. Typhimuriun* and *Staphylococcus aureus* and *Bacillus subtilis* with zone of inhagation ranging from 10-12. Our results suggest that further work is needed to locate the active principles from the various extracts or fractions and such efforts could result in the discovery of new compounds possessing a wide range of bioactivity for the treatment of infectious disease.

CONCLUSION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade. Plants are important for pharmacological research and drug development, not

only when bioactive phytochemicals are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. With latest advances in medicinal chemistry and knowledge of biosynthetic route for the development of lead compounds have opened new perspectives in the field of drug chemistry. There is a need to design new molecules from natural sources to combat lethal diseases. Thus plants are proving as potential source for drug discovery with anolides have known anti cancer activity which needs to be further explored. However, the integration of herbal medicine into modern medicinal practices, including treatments for infections and cancer, must contemplate the related issues of quality, safety and efficacy.

BIOLOGICAL ACTIVITIES

Anti-viral

Atropine inhibited only the growth of enveloped viruses independent of the nucleic acid content of the virus. The activity of atropine was checked by plaque reduction test and one step growth experiments. The test viruses included Herpes Simplex Virus, Influenza virus, New Castle Disease Virus, Sindbis, Vaccinia, Adenovirus, Japanese encephalitis Virus. Viruses were cultivated on primary chick embryo (CE), HeLa S3, primary monkey kidney cells (MK) (Yamazaki and Tagaya, 1980). Atropine also blocks the glycosylation of viral proteins of Herpes virus and hence the production of new infectious virus particles (virions). Virions formed in the presence of atropine are non infectious (Alarcon *et al.*, 1984).

Anticancer activity

Chemical investigation of a methanol extract of the flowers of *D. metel* by Pan *et al.* has led to the isolation of 10 new withanolides I-P (1-8), 1-10-seco-withametelin B (9) and 12 "-hydroxy-1, 10-seco-withametelin B (10) together with seven known withanolides. The structures were elucidated using spectroscopic data, while single crystal X-ray analysis was done for structure 9. The compounds were screened against various cancer cell lines including A549 (lung), BGC-823 (gastric) and K562 (leukemia). Compounds 1, 3, 4 and 6 exhibited cytotoxic activities with their IC₅₀ values ranging from 0.05 to 3.5 μ M.

Antiperspirant activity

Anticholinergic compounds have antiperspirant action (Mac Millan *et al.*, 1964). Various compounds have been studied and esters of cholinergic compounds have been found to be more effective. Scopolamine hydrobromide is found to be most effective due to its skin penetration ability.

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