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Research Article
Comparison of Different Solvents for Phytochemical Extraction Potential from *Datura metel* Plant Leaves

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Abstract

Background: The bioactive components present in the *Datura metel* plant are known to be responsible for its medicinal properties, to cure many diseases like asthma and bronchitis. However, the extraction method for these bioactive components is not yet standardized. The present study was undertaken to compare the effect of using different extraction solvents to extract the active components like alkaloids, flavinoids, saponins, steroids and tannins from the dried leaves of the *Datura* plant. **Methodology:** To achieve this, different extracts from the plant leaves were made using soxhlet apparatus. The extraction solvents used were distilled water, methanol, acetone, chloroform, ethyl acetate and hexane. Phytochemical estimations, total phenol concentration, flavonoid concentration and antioxidant activity have been evaluated to compare the efficiency of different extraction solvents. **Results:** The results shows that using methanol as an extraction solvent works best for the extraction of various active phytochemicals with flavonoid concentration of 16.48±0.22 mg of quercetin equivalent/100 g of extract, phenol concentration of 8.493±0.21 mg gallic acid equivalent/100 g of extract and 41.2±0.64% 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition which is significantly higher than chloroform which results in the minimum extraction with flavonoid concentration of 6.71±0.44 mg of quercetin equivalent/100 g of extract, phenol concentration of 0.196±0.02 mg gallic acid equivalent/100 g of extract and 15.3% 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition. **Conclusion:** To best of our knowledge this is the first report that directly compares the 6 extraction solvents for the extraction of active components from the *Datura* plant leaves and shows that methanol should be the solvent of choice.

Key words: *Datura*, extraction solvent, phytochemical, methanol, chloroform, ethyl acetate, hexane, acetone, phenol concentration, flavonoid concentration

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Datura metel is a plant which grows throughout the year and is known as Devil's Trumphet[1,2]. The plant was firstly described by the scientist Linnaeus in the year 1753. It can grow in the relatively hot and humid climate and is grown in all the parts of India. The plant has long flowers, which are white and purple in color. They are scented upto 6 inches. The leaves of the plant are broad in shape and dark green in color. They grow alternatively and are 10-20 cm long and 5-18 cm broad. The fruit of this plant is spiny capsule in nature having the thickness[3] of 4-10 cm. It is also used for ornamental purposes, for the decoration of the houses.

Datura metel plant is known to rich in many bioactive components like saponins, alkaloids, steroids, tannins, flavonoids and triterpenoids. Scopolamine is the major bioactive component and comes under the category of alkaloids[4]. These bioactive components are responsible for imparting medicinal properties to the plant and is thus useful in curing many human diseases like asthma and bronchitis[2]. It also helps in curing diabetes, heart diseases, insanity, epilepsy, skin disorders, fever and diarrhea[5,6]. The plant is also rich in withanolides and the flower of *Datura metel* is used in the treatment of pain[7]. It is known to possess hallucinogenic properties. The seeds of this plant are used in the place of opium and are also known to relieve dental pain as cavities can be cured by chewing *Datura* leaves[8,9].

Datura has wide applications in ayurvedic medicines too. Many constituents of Datura are used in ayurvedic preparations, which help in the treatment of hair fall and many other skin disorders[10]. The seeds of *Datura metel* have the potential of treating bleeding disorders[11]. The plant is also known to possess anti microbial and anti inflammatory activity[12]. Atropine, one of the important constituent of the *Datura metel*, dilates the pupil and also helps in the eye surgery[13]. *Datura metel* plant extract is also known to have herbicidal activity as methanolic extracts of the plant made from the dried leaves can remove unwanted weeds[14].

Inspite of the these important applications of *Datura metel* plant, reports have also demonstrated that at high dosage, *Datura metel* plant extract shows highly toxic effects which can be fatal and can cause mental disorders[4,15]. This effect of the plant extract is due to the presence of poisonous alkaloids in it[16]. Seeds are known to be most toxic even more than the leaves. The symptoms of the toxicity include hallucinations, fever, dilated pupils, headache, convulsions, coma and sometimes even the death of the person. It can also cause dryness in the mouth and urinary retention problems[15]. Due to this property, the dosage of *Datura metel* plant extract should be kept at minimum while manufacturing of medicines.

Although, the usefulness of this plant is well known in medicine, as antimicrobial, as insecticidal or herbicidal, the method of extraction of phytochemicals has not been characterized properly in the previous studies. The present study was undertaken to compare the efficiency of different extraction solvents in extracting the different phytochemicals from *Datura metel* dried plant leaves. The authors here for the first time report that methanol is the best extraction solvent and can be used for the extraction of phytochemicals from the *Datura metel* plant leaves.

MATERIALS AND METHODS

Materials: In this study, all the chemicals were provided by Hi-Media Co. including methanol, acetone, chloroform, ethyl acetate and hexane.

Collection of the plant sample and extract preparation: The plant was taken from the Herbal Garden of Lovely Professional University. The plant leaves were washed, shade dried and crushed to make the fine paste of the dried leaves. Extraction with different solvents like acetone, chloroform, distilled water, ethyl acetate, hexane and methanol were done using soxhlet apparatus. Briefly, for every 200 mL of the each solvent, 25 g of the crushed plant leaves powder was used for soxhlet extraction. After extraction for 3 consecutive days, the crude liquids were placed in water bath at 55°C for excess solvent evaporation.

Phytochemical screening: Biochemical tests were done to check the presence of different phytochemicals such as alkaloids, flavonoids, saponins, steroids and tannins in the mentioned *Datura metel* plant extract by following procedures mentioned in this study as:

- **Test for alkaloids:** About 10 mg of the each extract was taken and was dissolved in 2 mL of the Wagner's reagent. After dissolving the both, the appearance of reddish brown colored precipitates confirms the presence of alkaloids in the plant extract
- **Test for flavinoids:** About 10 mg of the each extract was taken and few drops of diluted NaOH was added to the each. The appearance of yellow colour which disappears or become colorless after adding few drops of diluted H_2SO_4 confirms the presence of flavonoids in the plant extracts

- **Test for saponins:** About 10 mg of the each extract was taken and diluted with 20 mL of distilled water. The test tubes were then shaken for 15 min by hand. Formation of foam on top of the test tube shows the presence of saponins in the plant extract
- **Test for steroids:** About 10 mg of the each extract was taken and 1 mL of concentrated H_2SO_4 was added to the each by the side walls of the test tube. Appearance of dark reddish green color confirms the presence of steroids in the plant extract
- **Test for tannins:** About 10 mg of the each extract was taken and was dissolved in 45% of the ethanol. The test tubes were then boiled for 5 min and 1 mL of 15% ferric chloride solution was added to each. The appearance of greenish to black color confirms the presence of tannins in the plant extract

Antioxidant activity: Blois[17] method was used to determine the free radical scavenging activity of the plant extract by using DPPH (2,2-diphenyl-1-picrylhydrazyl). Briefly, 0.2 mM DPPH solution was made using methanol. Ascorbic acid was taken as standard and 5 different concentrations (200, 400, 600, 800 and 1 mL) were taken to make the standard curve. The test samples were made by taking 10 µg of the each extract and dissolving in 2 mL of the mother solvent. About 1 mL of prepared DPPH was added to all the tubes. These tubes containing were kept in the dark for 60 min and the absorbance of all the samples were taken at 517 nm using spectrophotometer.

The percentage inhibition was calculated by using the formula:

$$[(A_{control}-A_{extract})/(A_{control})]\times 100$$

Total phenolic content in the plant: To determine the total phenolic content in plant extract, gallic acid was used as standard. The stock solution of the gallic acid was made by dissolving 10 mg in 100 mL of distilled water. Five different concentrations of the gallic acid was used as standard- 200, 400, 600, 800 and 1 mL and were used make standard curve. About 20 µL of the each extract was taken and volume adjusted to 2 mL using parent solvent. About 200 µL of FCR and 500 µL of 20% Na_2CO_3 were added to each tube. The reaction mixture was incubated for 1 h at room temperature and the absorbance was measured at 760 nm.

Quantification of the flavonoids in the plant: For the quantification of the flavonoids, quercetin was used as the standard. Different concentrations of the quercetin (200, 400, 600, 800 and 1 mL) were used to make standard curve. For test samples 10 µL of each extract was dissolved in 100 µL of particular parent extract. The final volume for the each sample was adjusted to 2 mL by adding 100 µL of potassium acetate, 100 µL of aluminum chloride and distilled water. The samples were then incubated for 30 min at the room temperature and the OD was taken at 470 nm.

Statistical analysis: Experimental values are expressed as Mean±SEM. Comparison of mean values between various groups was performed by one way-analysis of variance (one way-ANOVA). The $p<0.05$ was considered to be significant.

RESULTS

Extraction of active components from *Datura metel* plant using different solvents: The extracts from the dried leaves of the plant were made by using different solvents acetone, chloroform, distilled water, ethyl acetate, hexane and methanol for three consecutive days each in soxhlet apparatus. The extracted aqueous extracts were kept in the water bath at 55°C for evaporation of the solvent and the crude extract for the each solvent was obtained. After obtaining the extracts in the crude form, the percentage yield of the extracts were calculated (Table 1). Our results show that maximum percent yield is obtained in methanol extract (85.36%), followed by distilled water (78%), ethyl acetate (62.44%) and acetone (62.48%). Hexane and chloroform extraction results in minimum percent yield with 19.4 and 13.68% extraction, respectively. This highlights that methanol is efficient in extracting phytochemicals from the *Datura metel* plant leaves more than other extraction solvents.

Phytochemical screening: Phytochemical screening tests have been performed to detect the presence of bioactive components in the mentioned plant extracts. The results for these tests were as follows:

Table 1: Percentage yield of the extracts made from *Datura metel* plant using different extraction solvents

Solvents	Percentage yield (%)
Acetone	62.48
Chloroform	13.68
Distilled water	78.00
Ethyl acetate	62.44
Hexane	19.40
Methanol	85.36

- **Test for saponins:** The extracts of all the solvents were diluted with the distilled water and the test tubes were shaken for 15 min by hand and the formation of the foam on the upper layer of the test tube showed the presence of saponins in it. All the extracts of the different solvents, except chloroform showed the positive result for the presence of saponins. The tests were performed in triplicates. The chloroform extract showed the negative result may be due to improper solubility in the ferric chloride solution
- **Test for flavonoids:** All the extracts of the different solvents showed the positive result for the presence of flavonoids detected by the appearance of the yellow colour in all
- **Test for alkaloids:** The extracts of the different solvents showed the appearance of reddish brown color in the test tubes which confirms the presence of alkaloids in the plant extracts obtained using different solvents
- **Test for steroids:** All the extracts of the different solvents showed the appearance of dark reddish green color in the test tubes which confirms the presence of steroids in all the plant extracts
- **Test for tannins:** All the extracts of the different solvents, excluding chloroform showed the positive result for the presence of tannins in the plant due to the appearence of greenish black colour in the test tubes. The chloroform extract showed the negative result for the presence of tannins may be due to improper solubility

The results of these biochemical estimations are tabulated in Table 2.

Table 2: Results for the photochemical screening in different extraction solvents from *Datura metel* plant

Solvents	Alkaloids	Steroids	Flavinoids	Saponins	Tannins
Methanol	+	+	+	+	+
Hexane	+	+	+	+	+
Chloroform	+	+	+	-	-
Ethyl acetate	+	+	+	+	+
Distilled water	+	+	+	+	+
Acetone	+	+	+	+	+

+: Presence, -: Absence

Antioxidant activity: An antioxidant activity of all the plant extracts were observed using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ascorbic acid as the standard. A standard curve was made and DPPH activity was observed by measuring OD at 517 nm. It was observed that extracts from *Datura metel* plant which were formed using methanol, ethyl acetate, acetone and hexane shows the maximum antioxidant activity with 41.2±0.64, 40.8±0.69, 38±1.35 and 40.9±0.14% inhibition respectively followed by distilled water (31.9±0.23% inhibition) which is significantly higher than chloroform extract (only 15.3±0.14% inhibition) (Fig. 1, Table 3).

Total flavonoid quantification in the plant: Total flavonoid concentration was quantified by using quercetin as the standard and measuring OD at 470 nm. Using methanol as an extraction solvent results in the maximum flavonoid extraction with 16.48±0.22 mg of quercetin equivalent/100 g of extract followed by acetone, hexane and distilled water with 8.93±0.21, 6.04±0.89 and 3.6±0.20 mg of quercetin equivalent/100 g of extract, respectively. When the ethyl acetate was used as an extraction solvent, it results in the least extraction of flavonoids from *Datura metel* plant leaves (2.71±0.22 mg of quercetin equivalent/100 g of extract) (Fig. 1, Table 3).

Total phenolic content in the plant: Total phenolic content in the plant was estimated using gallic acid as a standard. The OD was taken at 760 nm. Maximum phenol concentration was observed when ethyl acetate (10.228±2.09 mg of gallic acid equivalent/100 g of extract) and methanol (8.493±0.92 mg of gallic acid equivalent/100 g of extract) was used as extraction solvent followed by acetone (2.049±0.08 mg of gallic acid equivalent/100 g of extract) and hexane (2.608±0.45 mg of gallic acid equivalent/100 g of extract). When chloroform and distilled water were used as extraction solvents, it results in the least extraction of phenols with 0.196±0.02 and 0.148±0.07 mg of gallic acid equivalent/100 g of extract obtained from *Datura metel* plant leaves (Fig. 1, Table 3).

Table 3: Comparison of flavonoid content, phenol content and DPPH activity from *Datura metel* plant extracts made with different solvents

Extracts	Flavonoid concentrations	Phenol concentrations	DPPH activity
Acetone	8.932±0.21*	2.049±0.08***	38.0±1.35*
Ethyl acetate	2.710±0.22	10.228±2.09***	40.8±0.69*
Methanol	16.480±0.22***	8.493±0.92***	41.2±0.64**
Hexane	6.044±0.89	2.608±0.45***	40.9±0.14*
Chloroform	3.60±0.44	0.169±0.02	15.3±0.14
Distilled water	6.710±0.20	0.148±0.07	31.9±0.23

Similar results were obtained in the three independent set of experiments. All the values were represented as Mean±SEM (n = 3), ***p<0.001, **p<0.01 and *p<0.05, vs extraction with distilled water

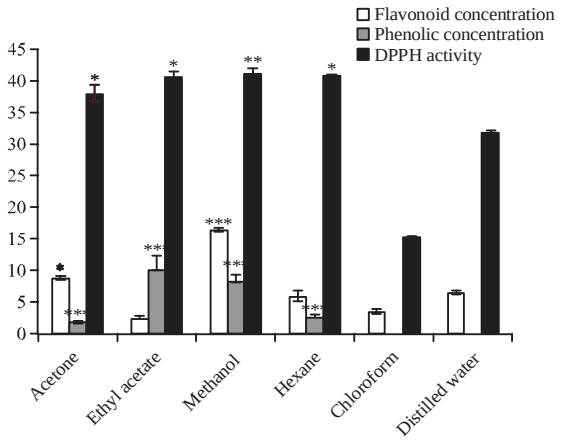

Fig. 1: Comparision of flavonoid content, phenol content and DPPH activity from *Datura metel* plant extract is made with different solvents. Similar results were obtained in the 3 independent set of experiments. All the values were represented as Mean±SEM. (n = 3), ***p<0.001, **p<0.01 and *p<0.05 vs extraction with distilled water

The maximum concentration of flavonoids, phenols and with parallel maximum antioxidant activity being achieved in methanolic extract highlights the efficiency of methanol over the other extraction solvents for extracting phytochemicals from the *Datura metel* plant leaves.

DISCUSSION

Datura metel is a well-known medicinal plant known to have health benefits against many diseases. These health benefits are mainly accounted to the presence of many active phytochemicals in various parts of this plant[5,6]. The present study was conducted with an objective to identify the best extraction solvent, which can be used to extract the maximum amount of the phytochemicals from the dried *Datura metel* plant leaves.

Qualitative biochemical estimations were conducted to detect the presence of different phytochemicals in the dried *Datura metel* plant leave's extracts obtained by using different solvents i.e., methanol. acetone, hexane, ethyl acetate, chloroform and distilled water. Our results highlights that all the extracts formed by using different solvents from *Datura metel* plant leaves contains phytochemicals like saponins, flavonoids, alkaloids, steroids and tannins. However, saponins and tannins were found to be absent in the extract made by using chloroform. It may be due to poor solubility of these phytochemicals in chloroform (Table 2). This signifies the inefficiency of chloroform to be used as phytochemical extraction solvent from *Datura metel* plant leaves. Previous reports have also shown that methanol and hexane extracts of *Datura metel* are more effective in controlling fungi *Macrophomina phaseolina*, which is responsible for causing charcoal rot disease in plants, as compared to chloroform extract[18].

A quantitative study was further conducted to detect the amount of the total flavonoids and total phenols in these plant extracts for direct comparison and have demonstrated that methanolic extract contains the maximum amount of flavonoids and phenols. Recently a study have evaluated the cytotoxic and antioxidant effects of extract made using methanol from different parts (seeds and fruit pulp) of *Datura metel* plant and have observed high concentration of flavonoids and phenol from extracts of *Datura* seeds which also parallel shows high antioxidant activity[19]. Further a comparative study have demonstrated the antioxidant and antimicrobial effects of *Datura metel* plant extracts made by using different solvents like butanol, methanol, ethyl acetate, hexane and chloroform from fresh and dried leaves and have shown that methanol extract possess the maximum antioxidant and antimicrobial activity[12]. The results from these studies are in line with our findings, which highlights that methanolic *Datura metel* plant extract possess the maximum concentration of flavonoid, phenols and also possess the maximum antioxidant activity (Fig. 1).

Previous studies have shown that *Datura metel* plant extract made with methanol is effective in treating gout by inhibiting xanthine oxidase[20] and also possess anti-proliferative activity against cancerous cell lines[21]. Reports have even shown that the methanolic extract of *Datura metel* plant possess antihelmintic activity and was able to suppress gastrointestinal nematodiasis in sheep and goat[22]. This highlights that due to the abundance of various phytochemicals in the *Datura metel* methanolic plant extract, it holds the great potential to treat various human diseases and has profound medical applicability. Reports have also shown the use *Datura metel* biomass for the phyto-remediation of industrial effluents[23]. This shows that methanolic extract of *Datura metel* being rich in phytochemicals can be effectively used for the remediation of industrial waste but further studies are required to confirm this.

CONCLUSION

Datura metel is well known for its medicinal properties. A comparative study has been conducted with an aim to achieve the best extraction solvent for the extraction of phytochemicals from *Datura metel* plant leaves. The results

from this study demonstrate that using methanol as extraction solvent results in the maximum extraction of flavonoids and phenols. Further, the concentration of flavonoids and phenols correlates very well with the anti-oxidant activity as methanol extract also showed the maximum antioxidant activity. This is followed by ethyl acetate, acetone and hexane as an extraction solvent as these solvents result in the moderate extraction of flavonoids, phenols and shows moderate antioxidant activity. Chloroform and distilled water results in the least extraction of various phytochemicals, may be due to poor solubility of these phytochemicals in chloroform and distilled water and should not be the solvent of choice. To best of our knowledge this is the first report that directly compares six extraction solvents and our results clearly demonstrates that methanol is the best extraction solvent for the extraction of various phytochemicals from the *Datura metel* plant leaves. This can be explored further.

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