INTRODUCTION

Daturametel L., with vernacular name “Dhatura” in Bengali-Indian language, is a shrub with evergreen branches. It is a perennial herbaceous plant, belonging to the Solanaceae family and often reaches a height of 1.5m. Leaves are simple, alternate, dark green, broadly ovate and shallowly lobed. Flowers are large, solitary, and trumpet-shaped with a sweet fragrance usually appreciated in the mornings and evenings, with a wide range of colours, ranging from white to yellow and light to dark purple. The flowers are bisexual and are pollinated by insects. The fruit is in the form of a capsule covered with short spines. Datura can tolerate average soil but prefers soil which is rich and moist or even very alkaline soil but hardly survives under shade. It prefers a warm temperature and is distributed in warmer regions of the world (Drake et al., 1996) [1]. Datura probably is of American origin and widely cultivated in all tropical and subtropical regions for its beautiful flower SD metel can also be found in East Asia or India, and is used in traditional Bangladeshi herbal medicine. In Traditional Chinese Medicine, the flowers of D. metel are known as Baiyantuo and are used for skin inflammation and psoriasis (Wang et al., 2008) [2]. In Ayurvedic medicine, seeds of the plant are used to treat skin rashes, ulcers, bronchitis, jaundice and diabetes (Agharkar et al., 1991) [3]. In Brazil, seeds are used for tea making which would serve as a sedative and flowers are dried and smoked as cigarettes (Agra et al., 2007) [4]. There are various species of Datura which are now cultivated for the production of secondary metabolites. Many different Alkaloids are found in the whole plant of Datura, which increased gradually with increase in age of the plant (Afsharypuor et al., 1995) [5]. Main constituents of the Datura plant are a huge number of tropane alkaloids (hyoscyamine, hyoscine, litorrino, acetoxytropine, valtropine, fastusine, fastusinine), a number of withanolides and various trigloyl esters of tropine and pseudotropine (Afsharypuor et al., 1995) [5]. Galystegines, the nortropane alkaloids with glycosidase inhibitory activity, have also been found in various Datura species (Ghani, 2003) [6]. The root contains higher amount of atropine compared to the other parts which is a common drug used to dilate pupil for eye examination. The aerial parts usually accumulated relatively higher amounts of scopolamine and relatively lower amounts of atropine as compared to the root of the plant (Afsharypuor et al., 1995) [5]. Thus the plant is a potential source of secondary metabolites which are possible sharp tools for medical cure.

MATERIALS AND METHODS

Collection of leaves

The leaves were collected from the field of Nalanchira, Thiruvananthapuram, Kerala (8.52 ° N, 76.94 ° E). The collected leaves were washed with water to remove dust and then washed leaves were shade dried for 14 d. After drying the leaves were ground into powder form using a mixer.

Extract preparation

Extraction procedure was done according to the method of Vikrant Arya et al. (2011) [7]. Extracts were prepared by using four solvents petroleum ether, ethyl acetate, aceton, methanol and ethanol.

Preliminary phytochemical screening

The phytochemical tests were done for analysing different chemical groups present in the extracts. These were done to find out the presence of bioactive chemical constituents such as alkaloids, flavonoids, saponins, steroids, terpenoids, tannins, glycosides, and amino acid compounds by the following procedure.

Test for alkaloids

3 ml of extract was added to 1% HCl and then allowed to steam bath. Few drops of Mayer and Wagner’s reagent (make: Sigma Aldrich) was added to the mixture. Turbidity indicates the presence of alkaloids.
Antioxidant activity

Determined (Meda et al., 2005) [8]. Gallic acid was used as standard.

Reducing power

The ability of extracts to reduce iron (III) was determined by the method of Yildirim et al [9]; four concentrations 25, 50, 75, 100 mg/ml of Daturametel solvent extracts were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide. Then, the mixture was incubated at 50°C for 30 min. 2.5 ml of Trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant solution was taken and mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride. Lascorhic acid was used as standard. Finally, the absorbance of all samples was measured at 700 nm. This was done in three replicates.

DPPH radical scavenging

The DPPH assay was done according to the method of Rivero-perez et al. (2008) [10]. The DPPH scavenging effects of the different extracts petroleum ether, ethyl acetate, acetone, methanol and ethanol were determined. Ascorbic acid was used as the standard. The blank contained 1 ml of distilled water. 1 ml of extract was taken at four concentrations of 25, 50, 75, 100 mg/ml in 4 test tubes. 1 ml of 0.2 mmol DPPH ethanol solution was added. The mixture was vortexed vigorously for 1 minute and kept in dark for 60 min. The absorbance of all samples was measured at 517 nm. This was done in triplicate. The percentage inhibition was calculated using the formula,

$$DPPH\text{ inhibition(\%)} = \%\text{ inhibition} = \left(\frac{\text{Absorbance of control–Absorbance of test sample}}{\text{Absorbance of control}}\right) \times 100$$

Antibacterial activity test organisms

Four pathogenic bacteria viz., Bacillus subtilis, Bacillus cereus, Salmonella typhi, and E. coli were collected from Pathogen laboratory, Medical College, Thiruvananthapuram. The cultures were sub cultured and maintained on nutrient agar slants in refrigerator at 4°C.

Inoculum preparation: Stock Cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of culture from the stock to test tubes of nutrient broth for bacteria and incubating for 24 h at 37°C and 25°C respectively. The cultures were diluted with fresh nutrient broth. Agar well diffusion method: The antibacterial screening of the crude extracts were evaluated by agar well diffusion method. After solidification of the medium, a well was made in the plates with sterile borer (5 mm). The extract compound 100μl was introduced into the well and the plates were incubated at 37°C for 24 h. All samples were tested in triplicates. The microbial growth was determined by measuring the diameter of the zone of inhibition.

### Table 1: Phytochemical analysis of Daturametel

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical compounds</th>
<th>Petroleum ether</th>
<th>Ethyl acetate</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Glucosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

### Table 2: Agar well diffusion method

<table>
<thead>
<tr>
<th>Extract in</th>
<th>Growth inhibition over different microbes (mm)</th>
<th>Bacillus subtilis</th>
<th>Bacillus cereus</th>
<th>Salmonella typhi</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetone</td>
<td>20</td>
<td>14</td>
<td>17</td>
<td>18</td>
<td>18</td>
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<tr>
<td>Methanol</td>
<td>21</td>
<td>17</td>
<td>16</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>Ethanol</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td>21</td>
<td>21</td>
</tr>
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</table>
Fig. 1: Total phenolic concentration of different solvent extracts of *Daturametel* leaves

Fig. 2: DPPH radical scavenging activities of different solvent extracts of *Daturametel* leaves

Fig. 3: Reducing power of different solvent extracts of *Daturametel* leaves

Fig. 4: Anti-microbial property exhibited by *Daturametel* leaf extracts in various solvents on *Bacillus subtilis*. A: Ethanol; B: Methanol; C: Acetone; D: Ethyl acetate; E: Petroleum ether
RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening result shows the alkaloids, flavonoids, saponins, tannins, glycosides and non-protein amino acids were present in leaf crude extracts of *Daturametel*, whereas steroids and terpenoids were absent in all the extracts (table 1). However, ethanol crude extracts showed positive test for alkaloids, flavonoids, saponins, tannins, glycosides, amino acids, but steroids and terpenoids were absent. Methanol crude extracts shows the presence of alkaloids, flavonoids, saponins, tannins and amino acids but glycosides, steroids and terpenoids were absent. In ethyl acetate crude extracts the chemical compounds such as alkaloids, flavonoids, saponins, glycosides and amino acids were present but tannins, steroids and terpenoids were absent. Petroleum ether crude extracts shows the presence for alkaloids, saponins, tannins and amino acids, whereas absent in all other screening test. Phytochemical constituents which are present in plant samples are known to be biologically active compounds and they are responsible for different activities such as antimicrobial, antioxidant, antiinflammatory, anticancer and anti-diabetic (Hossain and Nagooru; 2011) [11]. Different phytochemicals have been found to possess a wide variety of pharmacological activities, which may help in protection against chronic diseases. Tannins, glycosides, saponins, flavonoids, and amino acids have hypoglycemic and anti-inflammatory activities. Terpenoids, and steroids show analgesic properties and central nervous system (CNS) activities. Saponins are involved in plant defence system because of their antimicrobial activity (Ayoola et al., 2011) [12] and also possess hypocholesterolemic and anti-diabetic properties. These compounds are bio active compounds are alkaloids, saponins and amino acids-these were found in all four types of crude extracts. Flavonoids were found in ethanol, methanol and ethylacetate except petroleum ether extracts. Ethanol and methanol extracts show the presence of majority phytoconstituents. Many reports are available on flavanoid groups which exhibiting high potential biological activities such as antioxidant, anti-inflammatory, antiallergic reactions (Anyasor et al., 2010 [13]; Chao et al., 2002 [14]; Igbinosoa et al., 2009 [15]; Thilkerdecha, 2008 [16]).

Antioxidant activity

From the assay it’s proved that *D. metel* is a pool of antioxidants.

Total phenolic content

The amount of total phenolic compounds of crude extract was determined by using linear gallic acid (Y=8.7231X+0.087; R=0.9971). The total phenolic content ranges from 0.5-4.2 mg/ml (fig. 1). Among different types of extracts ethanol shows highest antioxidant activity whereas petroleum ether shows lowest antioxidant activity. In many plants, phenolic compounds show secondary metabolites with antioxidant and antibacterial activities. In many countries, 80% of people make use of medicinal plants for maintaining good health because of antioxidant property. Most medicinal plants contain higher phenolic compounds such as monophenols and polyphenols. Usually in many plants leaf shows the higher phenolic content (Pyo et al., 2004 [17]; Wong and Kitts, 2006 [18]). Many studies have proved that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents and hydrogen donors (Chang et al., 2001) [19].

DPPH radical scavenging

The photometric evaluation of the antioxidant activity of ethanol extract of *Daturametel* leaves shows good antioxidant capacity. The inhibition percentage of different extracts ranges from 6 to 53 mg/ml (fig. 2). In all extracts, ethanol showed maximum inhibition whereas petroleum ether showed minimum inhibition. Ethanol extract contain highest amount of total phenolics, was found to be most effective radical scavenger followed by methanol, ethyl acetate and petroleum ether extract. DPPH is used to evaluate the free radicals (Porto et al., 2000) [20]. Free radicals are involved in the process of lipid per oxidation which is considered as a major role in chronic diseases (Dorman et al., 2003) [21]. All extracts from *Daturametel* leaves exhibited a significantly greater hydroxyl radical scavenging activity than the ascorbic acid.

Reducing power

Many studies have indicated that the electron donation capacity of compounds is related with antioxidant activity. The reduction ability was estimated through Fe²⁺-Fe³⁺. Ethanol extract exhibits maximum reducing activity and Petroleum ether extract exhibits minimum activity. All extracts showed electron donation capacity. The higher absorbance value indicated that higher antioxidant activity. Ethanol extract contain highest amount of total phenolics and it is the most potent reducing agent (fig. 3). Relation between iron (III) reducing activity and total phenol content have been reported in the literature (Benzie et al., 1999) [22]; however the correlation may not be always linear (Yildirim et al., 2000) [9].

Anti-microbial activity

The results of present study showed that the selected plant *Daturametel* extracts were effective against the bacterial species tested. This can be used to treat *Bacillus subtilis, Bacillus cereus, Salmonella typhi* and *E. coli* (fig. 4); lists the inhibitory effect of different solvent extracts of *D. metel* on the test bacteria. It is observed that the leaf extract has more phytochemicals as compared to other plant parts where as it is very less in stem (Jamdhade et al., 2010) [23]. Current study agrees that the leaf extracts of *D. metel* inhibited the tested bacterial isolates. The higher inhibition on the bacterial species by ethanol extract of the leaves (26 mm), methanol leaf extract (21 mm) and acetonc leaf extract (20 mm) (table 2). According to Praanna and Raghunathan (2014) [24], in most cases the methanol and ethanol extracts exhibited higher antibacterial effects than the corresponding extracts.

CONCLUSION

High antioxidant activity was observed in ethanol extracts of *Daturametel* when compared to other extracts. The extracts of *D. metel* show the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, glycosides and non-protein amino acids. Ethanol contains highest amount of phenolic content and also exhibit strongest antioxidant capacity in the entire assay used. According to the phenolics result ethanol crude extract could be used as natural antibiotics for different diseases. The bioactive compounds from *D. metel* serve as good phytotherapeutic agent. The present antimicrobial of different crude extracts of *Daturametel* showed that the methanol, ethanol, and acetone from dry leaves shows highest zone of inhibition against the employed bacteria. This plant crude extracts in ethanol could serve as potential sources of antimicrobial agents and hold potential for being an antimicrobial drug.

CONFLICT OF INTERESTS

Declare none

REFERENCES

2. Wang XL. Accounting for autocorrelation in detecting mean shifts in climate data series using the penalized maximal t or F test. J Appl Meteor Climatol 2008;47:2423–44.
8. Medina A, Lamin GE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline

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