

# Phytoactives Screening and Evaluation of Antioxidant Potential of Thorn Part of *Euphorbia milii*

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**Abstract** - The present study aimed for phytoactives screening and assessment of antioxidant potential aqueous extract of thorn parts of *Euphorbia milii*. Thorn parts of *E. milii* were selected for phytoactives screening and subjected to successive solvent extraction by continuous hot soxhlet extraction with distilled water. The major phytochemicals found in aqueous extract of thorn parts of *E. milii* were, alkaloids, anthocyanin & betacyanin, flavonoids, phlobatannins, steroids, tannins, and terpenoids. The IC<sub>50</sub> values exhibited by aqueous extracts of thorn parts of *E. milii* was 211.38 µg/mL. In conclusion, study results showed that water concentrate of thorn parts of *E. milii* has wide variety of phytoactives. Antioxidant potential of watery concentrates of thorn parts of *E. milii* deduced that *E. milii* could be taken advantage of as imminent medication specialist of people drugs.

**Index Terms** - Antioxidants, *Euphorbia milii*, Thorn part, Phytoactives.

## I. INTRODUCTION

The genus *euphorbia* is the biggest genus of therapeutic plant. *Euphorbia milii* is generally known as “crown of thorn”.<sup>1,2</sup> The genus *E. milii* is the biggest variety of restorative plants broadly dispersed in tropical nations. Various types of *Euphorbia* are utilized for the treatment of different sicknesses like skin illnesses, gastrointestinal parasites and moles. It has been accounted for that *Euphorbia* has antiarthritis, anticancer, anticonvulsant, antidiabetic, hostile to dermatitis, calming, antimicrobial, cancer prevention agent, antispasmodic, antitumor, antitussive properties, hormonal and myelopoiesis properties.<sup>3</sup> A few types of *Euphorbia* have been generally utilized for the treatment of skin infections, gonorrhea, headache, gastrointestinal parasites and as mole fixes.<sup>4</sup> The class *Euphorbia* has been read up generally for its antiproliferative.<sup>5</sup>

Fungi of the family *Aspergillus* produce a poisonous substance called aflatoxin, which debases crops and causes human infections. Aflatoxin has even been ensnared as a contributing component in liver malignant cancerous diseases. *E. milii* flowers, when dried and handled as powder, hinder the development of *Aspergillus*.<sup>2</sup> Milin, a concentrate of *E. milii* latex, is a glycosylated serine protease. Since it is more steady than most proteases, it will be helpful to food processors and producers of cleansers who have been involving proteases in their activities.<sup>2,6</sup>

Literature search on phytochemical investigations of *E. milii* uncovered the presence of flavanoids, terpenoids, and tannins. Flavanoids are yellow shades, which happen in plant realm either in free state or as a glycosides or related with tannins. These are known as anthoxanthins.<sup>7</sup> With this foundation, the current study was attempted for phytochemical screening and assessment of antioxidant activities of aqueous extract of thorn parts of *E. milii*.

## II. MATERIALS AND METHODS

### Collection of plant material and processing

Aerial plant of *E. milii* were collected from natural habitats of Chikkaballapura districts of Karnataka State. Thorn parts were separated from *E. milii* plant material collected and sprayed with ethanol. Then shade dried at room temperature for 10 days. The dried thorn parts were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.<sup>2,8</sup>

### Extraction

Approximately 50 g of dried and coarsely powdered thorn parts of *E. milii* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water. All

the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

#### Phytochemical screening

Phytochemical screening was carried out on the aqueous extracts of thorn parts of *E. milii* by using standard procedure to detect constituents as described by Sofora,<sup>9</sup> Trease and Evans,<sup>10</sup> and Harborne.<sup>11</sup>

#### Alkaloids

Approximately 0.2g of aqueous extract of thorn parts of *E. milii* was warmed with 2% H<sub>2</sub>SO<sub>4</sub> (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

#### Tannins

The aqueous extract of thorn parts of *E. milii* in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride (FeCl<sub>3</sub>) was added. A dark green coloration indicates the presence of tannins.

#### Anthraquinone

Approximately 0.5g of aqueous extract of thorn parts of *E. milii* was boiled with 10% HCl for few minutes. The reaction mixture was then filtered and allowed to cool. Equal volume of chloroform (CHCl<sub>3</sub>) was added to each filtrate along with few drops of 10% NH<sub>3</sub> and heated. Rose-pink color formation was obtained which indicate the presence of anthraquinones.

#### Glycosides

About 0.6g of aqueous extract of thorn parts of *E. milii* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

#### Reducing sugars

The aqueous extract of thorn parts of *E. milii* was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

#### Saponins

About 0.2g of aqueous extract of thorn parts of *E. milii* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

#### Flavonoids

0.2g of aqueous extract of thorn parts of *E. milii* was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

#### Phlobatanins

About 0.5g of aqueous extract of thorn parts of *E. milii* was dissolved in distilled water and filtered. The filtrate was then boiled with 2M HCl solution. Red precipitates showed the presence of phlobatannins.

#### Steroids

2 mL of acetic anhydride was added to 0.5g of aqueous extract of thorn parts of *E. milii* and then added 2 mL of H<sub>2</sub>SO<sub>4</sub>. The change of color from violet to blue or green or red showed the presence of steroids.

#### Terpenoids

0.3g of aqueous extract of thorn parts of *E. milii* was mixed with 2 mL of chloroform (CHCl<sub>3</sub>) and 3 mL of concentrated 6M H<sub>2</sub>SO<sub>4</sub> was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

#### Anthocyanin and Betacyanin

To the 0.2g of aqueous extract of thorn parts of *E. milii*, NaOH (2N) was added and heated for 5 mins. at 100°C. Formation of bluish green colour showed the presence of anthocyanin and betacyanin.

#### Proteins and Amino acids

To the 0.3g of aqueous extract of thorn parts of *E. milii* few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue coloration indicates the presence of proteins.

#### Cardiac glycosides

Aqueous extract of thorn parts of *E. milii* was mixed with 1mL of glacial acetic acid (CH<sub>3</sub>COOH) and 5% ferric chloride (FeCl<sub>3</sub>) and then few drops of

conc.  $H_2SO_4$  was added. Greenish blue colour was observed which indicates the presence of glycosides.

#### Antioxidant assay

The modified literature protocol of Blois was used for antioxidant assay.<sup>12,13</sup> Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in distilled water. The control was also run which contains only distilled water. The hydrogen atom or electron donation abilities of each extracts and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control)  $\times$  100. The  $IC_{50}$  value was determined by using linear regression equation *i.e.*  $Y = Mx + C$ ; Here,  $Y = 50$ ,  $M$  and  $C$  values were derived from the linear graph trend line.

### III. RESULTS AND DISCUSSION

The major phytoactives detected in aqueous extracts of thorn parts of *E. milii* were, alkaloids, anthocyanin & betacyanin, flavonoids, phlobatannins, steroids, tannins, and terpenoids. The  $IC_{50}$  values exhibited by aqueous extracts of thorn parts of *E. milii* was 211.38 (Table 1 and 2).

Table 1: Photochemical screening of aqueous extracts of thorn parts of *E. milii*

| Chemical Components        | Thorn Part |
|----------------------------|------------|
| Alkaloids                  | +          |
| Anthocyanin and Betacyanin | +          |
| Antraquinone               | -          |
| Cardiac glycosides         | -          |
| Flavonoids                 | +          |
| Glycosides                 | -          |
| Phlobatanins               | +          |
| Proteins and Amino acids   | -          |
| Reducing sugar             | -          |
| Saponins                   | -          |
| Steroids                   | +          |

|            |   |
|------------|---|
| Tannins    | + |
| Terpenoids | + |

‘+’= presence and ‘-’=absence

Table 2: Antioxidant activities of aqueous extracts of thorn parts of *E. milii*

| S. No. | Extract    | $IC_{50}$ ( $\mu$ g/mL) |
|--------|------------|-------------------------|
| 1      | Thorn part | 211.38                  |

The fundamental screening and assessment of antioxidant activities of various parts of *E. milii* was attempted in the current study because of the way that they have been utilized in the people treatment against different illnesses and are rich wellsprings of optional metabolites and hydrocarbons. The current study uncovered that the phytochemical constituents of watery concentrate of thorn parts of *E. milii* incorporate alkaloids, anthocyanin & betacyanin, flavonoids, phlobatannins, steroids, tannins, and terpenoids. These discoveries are as per aftereffects of Rauf et al with the exception of steroids.<sup>2</sup>

Aqueous extract of thorn parts of *E. milii* showed antioxidant properties which might be because of the presence of phytoactives for instance flavonoids, terpenoids, tannins, and phenolic compounds.<sup>14-16</sup> The presence of phlobatannins in extracts proposes the diuretic property of the plant.<sup>17</sup> Flavonoids were found in watery concentrates of *E. milii* forestall oxidative cell harm demonstrating germicide, anticancer, calming impacts and mild hypersensitive properties.<sup>18</sup> Phenolic compounds present in water concentrate of thorn parts of *E. milii* are accountable for cell antioxidant properties.<sup>19</sup>

The outcomes got in our study are empowering as this study confirmed the wide assortment of auxiliary metabolites present in the watery concentrates of thorn parts of *E. milii* have shown impressive antioxidant properties. Along these lines, current study discoveries portrayed that thorn parts of the *E. milii* could be taken advantage of as people medication for the administration of different diseases.

### IV. CONCLUSION

Our study results showed that water concentrate of thorn parts of *E. milii* has wide variety of phytoactives. Antioxidant potential of watery concentrates of thorn parts of *E. milii* deduced that *E. milii* could be taken

advantage of as imminent medication specialist of people drugs. Besides, extra examinations are suggested to did to clarify the specific mechanism of action of different phytoactives present in *E. milii* against different illnesses.

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