Total flavonoid content and formulation antioxidant cream stem of *Jatropha multifida* L.

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**Abstract.** Free radical induced oxidative stress that influences the occurrence of various degenerative diseases such as cancer, coronary heart disease and premature aging. Stems of *Jatropha multifida* L are known to contain flavanoid compounds have antioxidant activity. A study has been carried out to determine antioxidant potential of stems of *Jatropha multifida* L. Initially, material was macerated gradually with ethanol. The extract obtained was filtered and evaporated. Determination of total flavanoid contents (TFC) using spectrophotometric methods. The antioxidant potential of this extract was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. In the DPPH radical-scavenging activities, the extract had the antioxidant activity (IC50 = 72 ± 0.01 µg/ ml). The results showed the extracts of *Jatropha multifida* L. could be considered as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration. The formulation comprises with 5% of extract and was formulated using fusion method. The evaluation of the formulated cream showed good results and can be good potential for cosmetic product development.

**Keywords:** 1,1-diphenyl-2-picrylhydrazyl (DPPH); total flavanoid contents (TFC); *Jatropha multifida* L, Cream

1. **Introduction**

Many herbal plants contain antioxidant compounds. Antioxidant compounds can protect cells against degenerative effects of Reactive Oxygen Species (ROS), such as singlet oxygen, superoxide, peroxyl, radicals, hydroxy radicals [1;2]. The concept of oxidative stress is that, when a balance between ROS production and antioxidant defenses is lost. ‘Oxidative stress’ result which through a series of events deregulate the cellular function and leads to various diseases. ROS-related disease such as aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neuro degenerative disease [3].

Flavonoid compounds are secondary metabolites commonly found in plants, useful in the defensive function against pathogens and radiation, and are directly involved in the antioxidant activity [4–6]. Antioxidants can be defined as any substance that, present in low concentration compared to an oxidized substrate, effectively delays or inhibits oxidation of the substrate [7]. For the food industry, it is also highly interesting to find new and safe antioxidants from natural sources. Although synthetic antioxidants are very effective and stable, they have limited use in many countries due to the possibility of causing adverse effects on human health [8,9].

Cosmetic products are used to protect skin against exogenous and endogenous harmful agents and enhance the beauty and attractiveness of skin [10]. The use of cosmetics not only developing an
attractive external appearance, but towards achieving longevity of good health by reducing skin disorders [11]. The plant parts used in cosmetic preparation should have varieties of properties like antioxidant, anti-inflammatory, antiseptic, emollient, antiseborrhatic, antikerolytic activity and antibacterial etc. Herbal products claim to have less side effects, commonly seen with products containing synthetic agents. The market research shows upward trend in the herbal trade with the herbal cosmetic industry playing a major role in fueling this worldwide demand for herbals [12].

2. Materials and Methods
2.1. Materials
The stem of *Jatropha multifida* L. was purchased from Karangjati, Kabupaten Semarang. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co, St Louis, USA. All other chemicals used were of analytical grade. *Jatropha multifida* L. was shown in Figure 1.

![Jatropha multifida L.](image)

Figure 1. *Jatropha multifida* L.

2.2. Extraction method
The dried stem (250 g) were macerated with absolute ethanol at room temperature for 24 h. The solution was filtered and concentrated under reduced pressure at 50°C, yielding 54,621 g crude ethanolic extract of *Jatropha multifida* L.

2.3. Qualitative Phytochemical Analysis
The stock solution was prepared from the crude extract and was dissolved in 10 ml of its own mother solvent. The stem extract was screened for terpenoids, tannins, saponins, flavonoids, phenolic and alkaloid as described by Trease and Evans. [13].

2.4. Total Flavonoid content.
The flavonoids content was determined by aluminium trichloride method using rutin as reference compound [14]. A volume of 150,0 mL of of extract is added to 20 ml water distillation and 1,5 mL 10% NaNO2 solution. The mixture was allowed to stand for 6 min, then 150 µL of aluminium trichloride (10%) was added and incubated for 5 min, followed by the addition of 20 mL of NaOH (10%). The final volume of the solution was adjusted to 50,0mL with distilled water. After 20 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm. The total flavonoids content was expressed as rutin.

2.5. Antioxidant assay
Crude ethanolic extract of *Jatropha multifida* L dissolved in ethanol were plated out in triplicate. The ethanolic DPPH (50 µM) solution was added to alternating columns of the test samples and ethanol was used for control of test samples. The percentage of decolourisation was obtained spectrophotometrically. The percentage of decolourisation was plotted against the concentration of the sample, and the IC₅₀ values were determined. The DPPH absorbance decreases with an increase in DPPH radical scavenging activity. Results were expressed as IC₅₀ concentration where 50% inhibition
of the DPPH radical is obtained. This activity is given as the percent of DPPH radical scavenged, which is calculated with the equation:

\[
\text{DPPH radical scavenging activity (\%) = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100\%}
\]

2.6. Preparation of Formulation

*Jatropha multifida* ethanolic stem extract was used to prepare the antioxidant cream. Composition of the cream were shown in Table 1.

**Table 1.** Composition of antioxidant cream

| Components                  | Amount (gr) |
|-----------------------------|-------------|
| Extract *Jatropha multifida* L. | 5           |
| Stearic acid                | 142         |
| Glycerin                    | 100         |
| Na. Tetraborat              | 2.5         |
| TEA                         | 10          |
| Methyl paraben              | 0.1         |
| Distilled water             | 750         |

The aqueous phase and oily phase components were heated separately up to 70°C and mixed uniformly using homogenizer by addition of methyl paraben and extract. Care was taken for even mixing, the remaining deionised water is added with continuous stirring until the mixture cools and formed as cream.

2.7. Evaluation of Antioxidant Cream

2.7.1. Physical Properties. The cream was observed for colour, odour and appearance. Determination of pH: The pH meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50ml of distilled water and its pH was measured. Determination of Emulsion Type (Dye test): The emulsion type was determined by using dye test. The scarlet red dye is mixed with the cream. Placed a drop of cream on a microscopic slide covers it with a cover slip and examined it under a microscope. If the disperse globules appears colourless the ground is red, the cream is oil in water type. The reverse condition occurs in water in oil type cream. i.e. the disperse globules appear red in the colourless ground.

2.7.2. Homogeneity. The formulations were tested for the homogeneity by visual appearance and by touch. After Feel Effect: Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

2.7.3. Viscosity. Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

3. Result and Discussion

The percentage yield and nature of the extracts were given in Table 2. The quantitative phytochemical analysis crude ethanolic extract of *Jatropha multifida* L showed the presence of terpenoids, tannins, saponins, flavonoids, phenolic and alkaloid compounds.

**Table 2.** Nature, Percentage Yield of the Extract

| Extract                            | Nature                  | Percentage Yield (%) |
|------------------------------------|-------------------------|----------------------|
| Crude ethanol extract (*Jatropha multifida* L. Stem) | Darkish Green; Semisolid | 21.65                 |
3.1. Total Flavonoids Content (TFC)
Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities [15]. The total flavonoids content of Jatropha multifida L extract was also determined using aluminium chloride colorimetric method. In this study, the total flavonoid stem of Jatropha multifida L were given in Table 3.

| Sample                                | Total flavonoid contents (mg/g) |
|----------------------------------------|---------------------------------|
| Crude ethanol extract (Jatropha multifida L. Stem) | 18,6067±0,041                   |

3.2. Antioxidant activity
DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. DPPH is stable free radical at room temperature and accepts an electron / hydrogen radical to become a stable diamagnetic molecule [16]. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity [17].

The relatively stable DPPH radical had been used widely to test the ability of compounds to act as free radical scavengers or hydrogen donors. This capability was used to evaluate antioxidant activity. Compounds with radical scavenger capacity are able to reduce DPPH radical using donor hydrogen atom to DPPH free radical based on the type and concentration of sample. Interaction of antioxidant compound with DPPH is based on transfer electron or hydrogen atom to DPPH radical and convert it to 1,1-diphenyl-2-picrylhydrazyl. The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which indicates the scavenging activity. The decrease of absorbance of DPPH radicals was measured at 517 nm.

The antioxidant activity of extracts was measured in terms of their efficient IC_{50} concentration corresponding to the sample concentration that reduced the initial DPPH absorbance of 50%. The IC_{50} value for Jatropha multifida L extracts determined by linear regression. IC_{50} value for the IC_{50} value of ethanol extracts and rutin was used as positive control of antioxidant were given in Table 4.

| Sample                                | IC_{50} (μg/ml) |
|----------------------------------------|-----------------|
| Rutin                                  | 28,50           |
| Jatropha multifida L. Stem extract     | 72,00           |

3.3. Evaluation of Antioxidant Cream
The formulated cream was shown in Figure 2.
The pH of the formulated creams was found to be 6.1 which is recommended as suitable pH in cosmetic skin cream formulations. The dye test confirms that the formulated creams were o/w type of emulsion cream. The formulated antioxidant cream were evaluated for several physicochemical tests and the results were shown in table 5. The formulated cream showed slight odour of the extract and green coloured cream. The formulated cream was not greasy after application to the skin. The formulated creams were easily removable by washing with tap water. The cream showed homogenous distribution of extract in the cream which was confirmed by visual examination.

Table 5. Evaluation of the formulated cream

| Parameter   | Formulation          |
|-------------|----------------------|
| Appearance  | green semisolid cream|
| Odour       | aromatic              |
| Homogeneity | Homogenous            |
| pH          | 6.1                   |
| Spreadability| Good                 |
| After feel  | Emollients and slipperiness |
| Removal     | Easily removed with tap water |
| Viscosity   | 289 cps               |

4. Conclusion

Based on the results of this study, the antioxidant capacities and total flavonoid content of *Jatropha multifida* L. stem considered as good sources of antioxidants as observed in DPPH scavenging assay. The study concludes that due to antioxidant activity, the topical application of the formulated cream from *Jatropha mufida* L stem extract will help in overcoming oxidative damage and can be considered as an alternative source in cosmetic industries.

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