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Assessment of Phenolic and Flavonoid Content of Six *Jatropha* plants Cultivated in Egypt and Evaluation their Anti-inflammatory and Antioxidant Properties

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Abstract: Alcoholic leaves extracts of six species belong to genus *Jatrtopha* (*J. integerrima* **Jacq**, *J. integerrima* **Rosea**, *J. multifida* **Linn**, *J. curcas* **Linn**, *J. gossypifolia* **Linn** and *J. pandurifolia* **Andrews**) were screened for their total phenolic and flavonoid contents along with evaluation of their *in-vitro* antioxidant and anti-inflammatory properties. The total phenolic and flavonoid contents were calculated as mg of gallic acid and quercetin equivalents (GAE & QE) / g DE by a colorimetric method utilizing Folin-Ciocalteu reagent and $AlCl_3$, respectively. Concerning the biological evaluation, DPPH radical scavenging assay and membrane stabilization method were used to determine the antioxidant and anti-inflammatory activities of plant extracts, respectively. The highest phenolic content was found in *J. gossypifolia* **L**. extract (11.21), followed by *J. curcas* **L**. (9.14), *J. integerrima* **Jacq** (5.01), *J. multifida* **L**. (4.26), *J. pandurifolia* **Andr**. (3.40) and *J. integerrima* **R.** (2.16). Moreover, *J. gossypifolia* **L**. recorded the highest flavonoid content (7.05), followed by *J. curcas* **L**. (6.33), *J. integerrima* **Jacq** (3.01), *J. pandurifolia* **Andr**. (1.25), *J. multifida* **L** (0.98) and *J. integerrima* **R.** (0.25). The *J. gossypifolia* **L**. has shown the best antioxidant activity (94.4%) followed by *J. curcas* **L**. (92.5%), *J. integerrima* **Jacq** (88.3%), *J. multifida* **L**. (73.5%), *J. pandurifolia* **Andr**. (66.9%) and *J. integerrima* **R.** (56.1%). However, the highest anti-inflammatory activity was exerted by *J. gossypifolia* **L**. (99.8%), followed by *J. integerrima* **R.** (89.6%), *J. curcas* **L** (82.5%), *J. multifida* **L**. (66.2%), *J. pandurifolia* **Andr**. (60.5%) and *J. integerrima* **Jacq**. (47.1%). The findings demonstrated that the investigated *Jatropha* species could be optimized as potential sources of natural antioxidant and anti-inflammatory agents.

Keywords: *Jatropha*, Phenolic, Flavonoid, Colorimetric, Antioxidant, Anti-Inflammatory.

1. INTRODUCTION

The *Jatropha* is a genus belongs to the Euphorbiaceae or spurge family, which comprising around 175 species of herbs, trees, shrubs and subshrubs that are originated in tropical regions in Africa and America [1]. It is subdivided into two subgenera; subgenus *Jatropha* which is mostly grows in India, America and Africa, and subgenus *Curcas*, which is originated from Texas, Mexico and Arizona[2]. Some species were successfully acclimated in Egypt including *J. integerrima* **Jacq**, *J. integerrima* **R.**, *J. multifida* **L.**, *J. curcas* **L.**, *J. gossypifolia* **L.**, and *J. pandurifolia* **Andr**, which were selected in the current study. Many species of *Jatropha* are utilized in folk medicine in many countries. *J. curcas* leaves decoction is used for treatment of mouth sores in Ghana[3], while that of *J. gossypifolia* is employed for the treatment of diarrhea in India and Barazil[4]. Leaves of *J. integerrima* **Jacq,** are commonly utilized as purgatives[5], and *J. multifida* fruits are applied topically in case of skin diseases in Cambodia[6]. Many of these traditional applications are scientifically supported[7]. Besides validating possible medicinal value, a wide range of diterpene skeletons was reported as the major natural products class in *Jatropha*[7, 8]. Other types of natural products were reported in *Jatropha* plants such as

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triterpenoids (sterols, alcohols and hydrocarbons), phenolics such as flavonoids[9], lignans, coumarins, tannins[10], and glucosinolates[11], which make them of high interest for the agricultural, nutritional and pharmaceutical industries. The presence of tannins such as gallic acid and pyrogallol, flavonoids and isoflavonoid within side the *J. curcas* (kernel meal) can also additionally make contributions to the antioxidant interest of extracts. These compounds had been recognized as the most important compounds concerned in antioxidant activity[12]. There are a few reviews posted on medicinal benefits from *J. curcas* species[13-17], there's a vital need for an inclusive overview that covers the healing and toxicological capacity of this species. Flavonoids and phenols, found in *Jatropha curcas*, are the principle class of polyphenolic compounds in plants[18]. It reveals interest towards gram-positive bacteria[19] and *Streptococcus mutans*[20]. Because of the antioxidant activity *in vivo* and *in vitro* research, phenolic compounds have recently gained a lot of attention. Phenolics and flavonoids can be extracted using a variety of methods, including acid, alkali, and enzymatic hydrolysis, to produce powerful bioactive compounds that can be used as anticancer, cardioprotective, and anti-inflammatory agents[21].

2. METHODS

2.1. Collection and drying of plant material

During June (2018), leaves of *J. integerrima* **Jacq**, *J. integerrima* **R.**, *J. multifida* **L.**, *J. curcas* **L.**, *J. gossypifolia* **L.**, and *J. pandurifolia* **Andr.** were collected from plants cultivated in the Orman Garden, Giza, . The plants were discovered and described by Dr. Therese Labib Youssef, Ex-Manager and Taxonomist of Botanical Orman Garden, Giza, Egypt. A voucher specimen (Reg. No. Ji-I, Ji-II, Jm-III, Jc-IV, Jg-V, and Jp-VI respectively) of plants was deposited within Pharmacognosy Department herbarium, Faculty of Pharmacy (Girls), Al Azhar University, Cairo, Egypt. The material was dried in a well aerated shaded place, powdered and saved separately in tightly closed containers.

2.2. Plant extract preparation

The dried powdered leaves (500g, /sample) of six *Jatropha* species macerated with absolute ethanol (3x1L), then filtered using whatman No.1 papers, and dried under vacuum using rotatory evaporator (at 50 °C) (Buchi, G. Switzerland). The concentrated extracts were kept separately in tightly closed containers and dry place at low temperature (- 4 °C).

2.3. Total Phenolic Content determination

TPC was established quantitatively in all extracts by Folin-Ciocalteu reagent as mentioned in **(Meda A *et a*l., 2005)** [22].

2.4. Total Flavonoid Content determination

TFC was quantitatively calculated using a conventional approach described in the literature **(Chang CC *et al*., 2002)** [23].

2.5. In vitro anti-inflammatory activity evaluation

Individually, the anti-inflammatory potential of the alcoholic extracts of the six species of *Jatropha* leaves under study was calculated by the Membrane stabilization method which mentioned by (**Anosike CA *et al*., 2012**) [24].

2.6. In vitro antioxidant activity evaluation

The radical-scavenging percentage of DPPH (2, 2'-diphenyl-1-picrylhydrazyl) was calculated using a standard published procedure **(Cheng Z et al., 2006)** [25].

3. RESULTS

3.1. Total Phenolic Content

As shown in **Fig.1**, *J. gossypifolia* **L**. has the highest phenolic content (11.21 mg GAE/g DE), followed by *J. curcas* **L**. (9.14 mg GAE/g DE), *J. integerrima* **Jacq** (5.01 mg GAE/g DE), *J. multifida* **L**. (4.26 mg GAE/g DE), *J. pandurifolia* **Andr**. (3.40 mg GAE/g DE), and *J. integerrima* **R.** (2.16 mg GAE/g DE).

3.2. Total Flavonoid Content

The highest TFC content was found in *J. gossypifolia* **L**. plant (7.05 mg QE/g DE), followed by *J. curcas* **L**. (6.33 mg QE/g DE), *J. integerrima* **Jacq** (3.01 mg QE/g DE), *J. pandurifolia* **Andr**. (1.25 mg QE/g DE), *J. multifida* **L**. (0.98 mg QE/g DE), and *J. integerrima* **R.** (0.25 mg QE/g DE), as shown in **Fig 2**.

3.3. *In vitro* antioxidant study

The DPPH radical scavenging behavior of ethanol extracts of selected *Jatropha* species was assessed at various concentrations in comparison to ascorbic acid, which served as a reference drug (**Fig. 3)**. When compared to ascorbic acid (99.9 %), *J. gossypifolia* **L**. has the highest antioxidant activity (94.4 %), followed by *J. curcas* **L**. (92.5 %), *J. integerrima* **Jacq** (88.3 %), *J. multifida* **L**. (73.5 %), *J. pandurifolia* **Andr**.(66.9%), and *J. integerrima* **Rosea** (56.1 %). In a dose-dependent manner, the percent of antioxidant activity is increased. In addition, IC_{50} was calculated and expressed as µg/ml

In contrast to ascorbic acid, which had the lowest IC_{50} (9.16 µg/ml), followed by *J. gossypifolia* **L**. (13.44 µg/ml), *J. curcas* **L**. (19.04 µg/ml), *J. integerrima* **Jacq** (41.15 µg/ml), *J. multifida* **L**. (225.60 µg/ml)

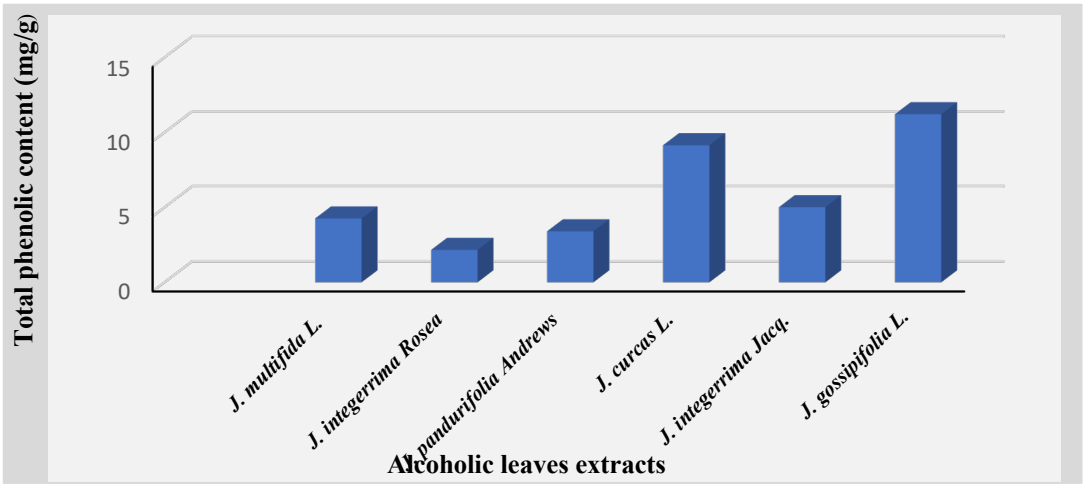

Figure 1: Quantitative determination of the TPC in plant extracts (mg GAE/g DE)

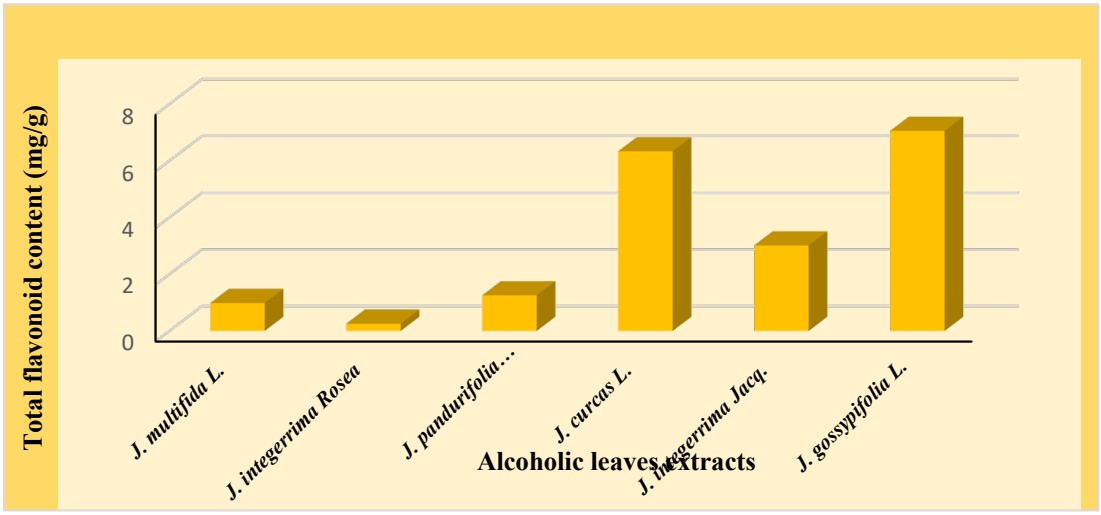

Figure 2:Quantitative determination of the TFC in plant extracts (mg QE/ g DE)

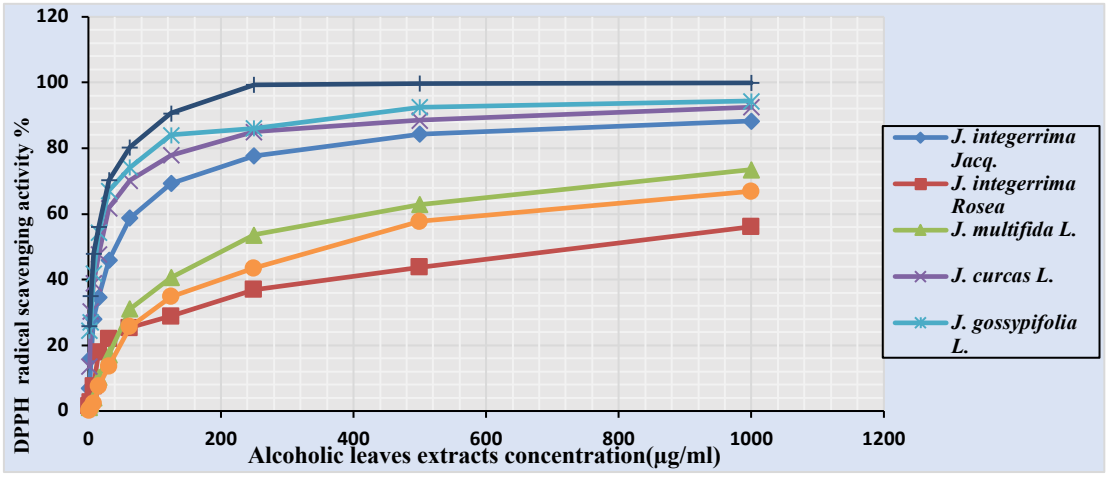

Figure 3: DPPH scavenging assay of different species of Jatropha ethanol extracts

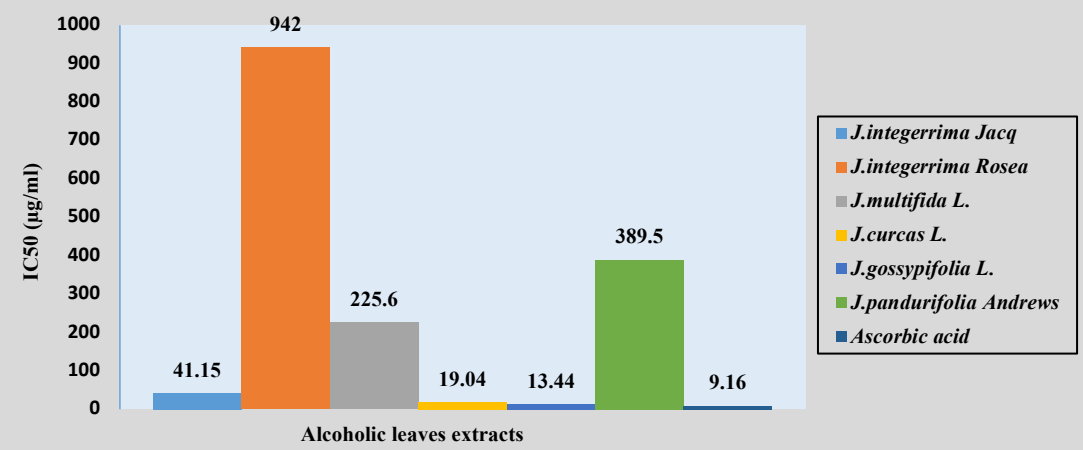

Figure 4: Fig. 4. IC50 (µg/ml) of ethanol extracts against ascorbic acid

3.4. *In vitro* Anti-inflammatory study

The percentage of anti-inflammatory activity for different Jatropha extracts was 99.8, 89.6, 82.5, 66.2, 60.5, and 47.1 for *J. gossypifolia* **L.**, *J. integerrima* **R.**, *J. curcas* **L**, *J. multifida* **L.**, *J. pandurifolia* **Andr**, and *J. integerrima* **Jacq**, respectively, according to the results in **Table 1**. As a result, we conclude that the Jatropha species studied in this analysis have anti-inflammatory properties and thus can be used as a natural remedy to reduce inflammatory injury and tissue damage.

3.5. TPC and antioxidant activity relationship

The order of the tested samples' antioxidant activities corresponds that of their total phenolic contents; this could be referred to as the correlation between antioxidant properties and total phenolic contents as shown in **Fig. 5**.

The correlation coefficient (r) is calculated by the following equation:

$$\frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2\sum(y-\bar{y})^2}}$$

Where: $\bar{x}$ and $\bar{y}$ are the sample means average (antioxidant activity) and average (total phenolic content), respectively. So, r = 0.960, which indicates a strong positive correlation between TPC and antioxidant activity (DPPH radical scavenging %).

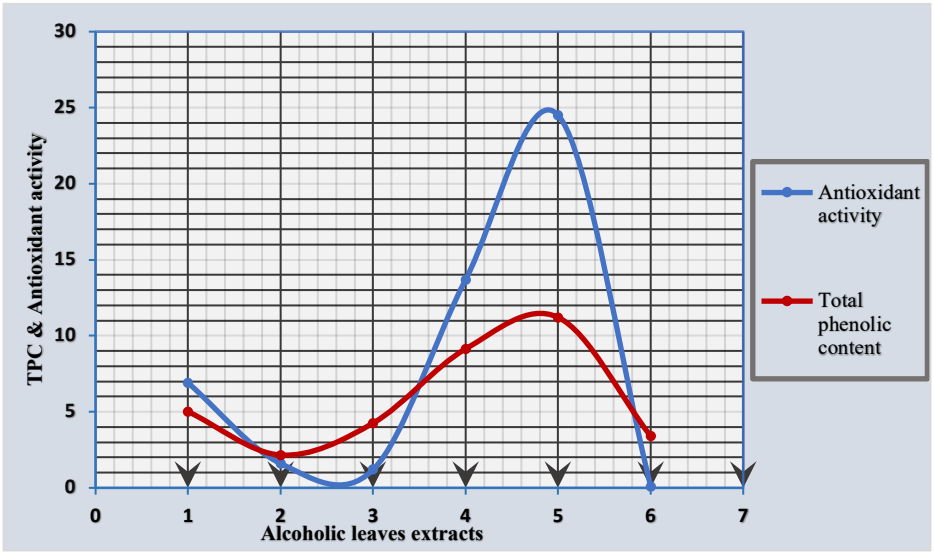

Figure5 : The correlation between TPC and antioxidant activity

Table 1:. The Effect of Jatropha extracts on HRBC hemolysis and membrane stabilization

Sample	Conc. (µg/ml)	Hypotonic absorbance mean	Sample with isotonic solution absorbance.	Hemolysis inhibition %
Control		1.45		0
***J. integerrima* Rosea**	1000	1.114	1.075	89.6
	800	1.077	0.998	82.5
	600	1.019	0.789	65.2
	400	1.012	0.725	60.4
	200	1.002	0.678	58.0
	100	0.936	0.524	55.5
***J. ntegerrima* Jacq.**	1000	0.88	0.243	47.2
	800	0.977	0.104	35.1
	600	1.035	0.095	30.6
	400	1.055	0.084	28.9
	200	1.061	0.071	28.2
	100	1.102	0.002	24.0
***J. multifida* L.**	1000	0.842	0.531	66.2
	800	0.843	0.411	58.4
	600	0.846	0.352	55.0
	400	0.832	0.299	53.7
	200	0.807	0.22	52.3
	100	0.854	0.052	42.6
***J. gossypifolia* L.**	1000	0.985	0.984	99.8
	800	0.917	0.9	96.9
	600	0.917	0.821	84.7
	400	0.894	0.778	82.7
	200	0.873	0.721	79.1
	100	0.733	0.52	77.1
***J. curcas* L.**	1000	0.911	0.797	82.5
	800	0.855	0.657	75.0
	600	0.824	0.591	72.9
	400	0.756	0.458	70.0
	200	0.708	0.355	67.8
	100	0.706	0.241	61.5
***J. pandurifolia* Andrews.**	1000	0.798	0.373	60.5
	800	0.868	0.311	51.1
	600	0.879	0.245	47.4
	400	0.896	0.195	44.1
	200	0.97	0.095	35.4
	100	1.032	0.034	29.5

4. DISCUSSION

In the current study, we observed that the alcoholic extract of different six *Jatropha* species leaves under investigation had a relatively high variable content of phenolic and flavonoid compounds, which responsible for their main biological activity. The most promising antioxidant *J.*species were *J. gossypifolia* **L**, *J. curcas* **L**, *J. integerrima* **Jacq**, and *J. multifida* **L**, respectively. They also had the same order in their total phenolic content, which encourages us to correlate their total phenolic content with their antioxidant potential. The results of the correlation study indicate a strong positive correlation, which proved that the antioxidant activity depends particularly on the concentration of TPC. Many types of researches

targeting the biological activities of phenolics that are reported as strong antioxidants and powerful radical scavenging agents[26]. The antioxidant potential of phenolic compounds is principal because of their redox behavior, which potentiates their reducing properties and oxygen quenching activity[27]. Phenolic compounds also are known to play a vital role to protect lipids against peroxidation and inactivate many types of oxidizing enzymes[28].

On the other side, we found that the anti-inflammatory order of *J. integerrima* **R.** alcoholic leaves extract differed from that of antioxidant activity, which means that this species contains another substance that responsible for its anti-inflammatory activity, so it needs further chromatographic and biological studies to prove that. The anti-inflammatory technique used in this study was depending on the fact that, the human red blood cell (HRBC) membranes are almost like lysosomal membrane components[29]. The hemolysis which caused by the hypotonic solution is due to the accumulation of fluids into the cell resulting in rupturing of the HRBC membrane. Injury to RBCs membrane will make the cell liable to other damages via free radical as in the case of lipid peroxidation[30]. Membrane stabilization results in the control of the serum protein and fluids release within the tissues which is stimulated by most inflammatory mediators[31]. The inhibition of hypotonicity was considered as a measure of the anti-inflammatory potential of ethanol extracts of *J.* species. Phenolic acids might have polyphyletic effects on immunomodulation, we suggest that some flavonoids in the selected species in the current study reported having anti-inflammatory activities. The dietary intake of some flavonoids can decrease the inflammation caused by endotoxin in the tissues of the liver and intestine[32].

5. CONCLUSIONS

We can conclude that the leaves of *J.* species used in the current study; are natural sources rich with phenolic constituents, so they are considered as a natural antioxidant and anti-inflammatory remedies. Consequently, further phytochemical investigations and chromatographic studies should be conducted for the extracts of the most promising *J.* species, which are rich in phenolics, aiming at the isolation of phenolic constituents in pure form for clinical studies.

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Conflicts of Interest: The authors declare no conflict of interest

Author Contribution: Shimaa M. Khalifa performed the extraction and wrote the paper. Mohamed Marzouk and Hala Sh. Mohammed participated in the supervision of the work and revision of the paper. Amal H. Ahmed and Ahmed M. Metwally participated in the supervision of the work.

List of Abbreviations: TPC: Total Phenolic Content; TFC: Total Flavonoid Content; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; DE: Dry Extract; DPPH: 2, 2'-diphenyl-1-picrylhydrazyl; ROS: Reactive Oxygen Species; $IC_{50:}$ Median Inhibition Concentration; *J.: Jatropha;* Jacq.: Jacquin; Andr.: Andrews; R.: Rosea; L.: Linnaeus; HRBC: Human Red Blood Cell.

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