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EVALUATION OF PHYTOCHEMICAL AND ANTIMITOTIC POTENTIAL OF *Ruellia prostrata* LEAF EXTRACT BY ONION ROOT MODEL

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ABSTRACT

Like most other plants *Ruellia prostrata* contain various secondary metabolites with great potentials. The present study deals with the phytochemical profile and evaluation of antimitotic activity of leaf extracts of *Ruellia prostrata* leaves using *Allium cepa* root tip cells. The phytochemical characters of aqueous and ethanol extract of *Ruellia prostrata* leaves were investigated. Tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones and polyphenol while emodins, anthocyanins were absent in both extract. Alkaloids and glycosides were present only ethanol extract. Coumarin was present only aqueous extract. Significant concentration of flavonoids and total phenol were present in *Ruellia prostrata* leaves extract. The ethanolic extract of *Ruellia prostrata* leaves had excellent anti-mitotic activity to be concluded presence of anti-cancer properties was reported in our study.

Keywords: *Ruellia prostrata*, phytochemicals, qualitative, quantitative and anti-mitotic.

INTRODUCTION

Medicinal plants are a reservoir of biologically active compounds with therapeutic properties that over time have been reported and used by diverse groups of people for the treatment of various diseases (Prasathkumar *et al.,* 2021). Natural products, especially those derived from plants, have been used to help mankind sustain human health since the dawn of medicine. Traditional medicine has been in existence since time immemorial and has been well accepted and utilized by people throughout history. Since ancient times, plants have been an exemplary source of medicines. Plants rich in bioactive phytomedicine compounds such as alkaloids, flavonoids, tannins and polyphenols have been used to cure illnesses because of their various pharmacological properties (Aye *et al.,* 2019). The antimitotic are considered as mitosis blockers and consequently, cell death inducers. Antimitotic agents widely used in chemotherapy, target exclusively proliferative cells and commonly induce a prolonged mitotic arrest followed by cell death via apoptosis (Umadevi *et al.,* 2013). The general principle of the mechanisms of mitosis are best and most easily studied in the actively growing region of plants such as a shoot or root apex. Mitosis is the characteristic type of cell division which keep the genetic continuity and integrity of organisms and preserves their somatic chromosome value. It is also known as equational division. Since the daughter cells formed are genetically and morphologically identical. Mitosis involves two closely related processes, karyokinesis and cytokinesis. Karyokinesis include 5 sub stages; prophase,

prometaphase, metaphase, anaphase and telophase (Mitsuhiro Yanagida, 2014).

Mitosis is the division of somatic cells into two daughter cells. Durations of the cell cycle and mitosis vary in different cell types. An elevated mitotic index indicates more cells are dividing. In cancer cells, the mitotic index may be elevated compared to normal growth of tissues or cellular repair of the site of an injury (Williams and Omoh, 1996). The mitotic index is therefore an important prognostic factor predicting both overall survival and response to chemotherapy in most types of cancer. The antimitotic activity is mostly screened using Allium cepa root meristematic cells which have been used extensively in screening of drugs with antimitotic activity (Srinivas *et al.*, 2010; Pardesi Goldee *et al.*, 2008). Keeping in view in the present study to analysis the phytochemical and evaluation of antimitotic activity of *Ruellia prostrata.*

MATERIALS AND METHODS

Collection of plant

The leaves of *Ruellia prostrata* were collected from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from an herb.

Preparation of extract

The collected *Ruellia prostrata* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with ethanol and aqueous for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used

Preliminary phytochemical analysis

Preliminary phytochemical screening was carried out by using standard procedure Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The total phenol contents were estimated by the method of Edeoga *et al*., (2005). The total flavonoid was determined by the method of Bohm and Kocipai-Abyazan (1994). Saponin is determined by the method of Obadoni and Ochuko (2001). Steroids content in the sample was evaluated using Buljet's reagent as described by Attarde Daksha *et al*., (2011). Estimation of total terpenoid content (Ferguson, 1956). Histochemical tests were carried out by method of John Peter Paul, (2014); Gersbach *et al.*, (2001).

Evaluation of antimitotic activity using *Allium cepa* roots

Antimitotic activity study was conducted as per the methods reported by previous workers with modifications (Grant, 1982; Fiskesjo, 1988; Shweta *et al.* 2014).

RESULTS AND DISCUSSION

The phytochemical characters of aqueous and ethanol extract of *Ruellia prostrata* leaves were investigated and summarized in Table 1. Tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinones and polyphenol while emodins, anthocyanins were absent in both extract.

Alkaloids and glycosides were present only ethanol extract. Coumarin was present only aqueous extract. Quantitative analysis of *Ruellia prostrata* showed that rich amount of total phenol (217.62 ± 12.23mg/gm), terpenoids (71.47 ± 2.20mg/gm), flavonoids (132.85 ± 7.75mg/gm), saponin (46.79 ± 3.27mg/gm) and steroids (37.43 ± 1.22mg/gm) were presented (Table 2).

Secondary metabolites are reported to have many biological and therapeutic properties.

Table 1: Qualitative analysis of phytochemicals in *Ruellia prostrata* leaves

S. No	Phytochemicals	Aqueous extract	Ethanol extract
1	Tannin	+	++
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	++	++
5	Terpenoids	++	+
6	Triterpenoids	++	+
7	Alkaloids	-	+
8	Anthroquinone	++	+
9	Polyphenol	++	++
10	Glycoside	-	++
11	Coumarins	++	-
12	Emodins	-	-
13	Anthocyanins	-	-

(-) Indicates absent; (+) Indicates Presence; (++) Moderately present

Table 2: Quantitative phytochemical analysis of *Ruellia prostrata* leaves extract

Phytochemicals	Results (mg/gm)
Total phenol	217.62±12.23
Flavonoids	132.85±7.75
Saponins	46.79±3.27
Terpenoids	71.47±2.20
Steroids	37.43±1.22

Values are expressed as mean ± SD for triplicates

Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah *et al.*, 2012; Velavan Sivanandham, 2011). The phytochemical analysis of various extract (Aqueous and ethanol) of *Ruellia prostrata* leaves was investigated. Among the various extracts, ethanol extract showed the presence of rich compounds in leaves extract. This research agreed with work reported by Arul and Saravanan, (2017); Velavan, (2015)

Histochemical analysis

In the present study, *Ruellia prostrata* leaves powder were treated with specific chemicals and reagents to identify the phytochemicals under the light microscope. This results further confirmed the presence of phytochemicals in *Ruellia prostrata* leaves (Table 3 and Plate 1). The results showed that the black, yellow, reddish brown, green, blue green, orange and brown colours indicate the presence of tannin, flavonoids, alkaloids, steroids, poly phenol, terpenoids and glycoside respectively in leaves of *Ruellia prostrata*. Histochemical studies are helpful in drug adulteration and systematic hierarchy of taxon.This research agreed with work reported by John Peter Paul, (2014) attempt was taken for histochemical and fluorescence analysis of *Turbinaria ornata* (Turner).

Table 3: Histochemical analysis of *Ruellia prostrata* leaves powder

Phytochemicals	Colour observation	Result
Tannin	Black	++
Flavonoids	Yellow	++
Alkaloids	Reddish brown	++
Steroids	Green	++
Poly phenol	Blue green	++
Terpenoids	Orange	++
Glycoside	Brown	++

Note: (+) Presence; (++) present with high intensity of the colour

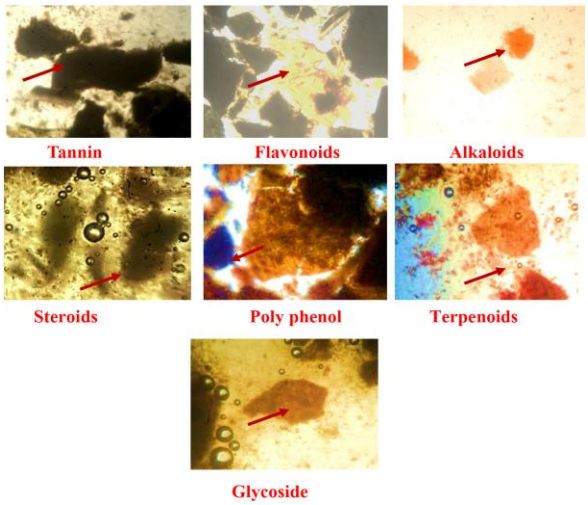

Plate 1: Histochemical analysis of *Ruellia prostrata* leaves powder

Histochemical analyses of the plant were carried out using light microscopy and fluorescence study was analyzed by UV lamp. Histochemical tests showed positive reaction to phenol compounds, polyphenol and tannin in the thallus. Fine powder and different solvent extracts of *Turbinaria ornata* obtained using petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light.

Antimitotic activity in *Ruellia prostrata* leaves extract

The aim of this work was investigate the antimitotic activity of *Ruellia prostrata* leaves extract (Plate 2, 3). The ethanolic extract of *Ruellia prostrata* produced root decay and decreased the root length and root number significantly at 96 h as compared to control ($p<0.05$). The average root length in control, 100, 200, 300, 400µg/mL and 500µg/mL of *Ruellia prostrata* was 0.57, 0.95, 0.73, 0.63 0.42 and 0.29mm at 96 hr respectively. The root numbers in control, 100, 200, 300 400µg/mL and 500µg/mL of *Ruellia prostrata* was 16, 14, 12, 9, 6 and 4 at 96 hr respectively. The mitotic index at 100, 200, 300 400µg/mL and 500µg/mL of *Ruellia prostrata* was 83.95, 72.79, 43.01 and 11.95% at 96 hr respectively. The highest dose as 500mg/mL of *Ruellia prostrata* has significant activity in root length, number and mitotic index (Table 4, 5 and Figure 1). The rate of tumor growth is dependent upon a balance between the rates of cell proliferation and apoptosis. Apoptosis is a programmed cell death, as influenced by phytosterol (Awad *et al*., 2000).

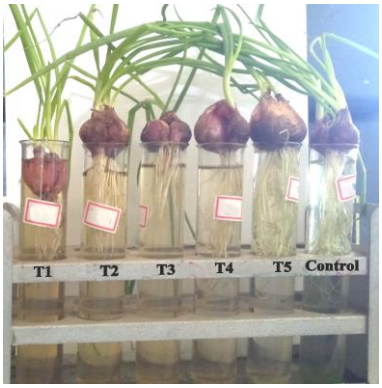

Plate 2: Evaluation of antimitotic activity in *Ruellia prostrata* leaves extract using *Allium cepa* L. root

Table 4: Effect of *Ruellia prostrata* on root length and root number of *Allium cepa* roots.

Groups	Mean root length (mm)			Mean root Number (s)		
	Before treatment	After treatment	Average root growth	Before Treatment	After Treatment	Average root number
Group I (Water control)	4.32	4.89	0.57	33	49	16
P. prostrata 100µg/ml	5.11	6.06	0.95	67	81	14
P. prostrata 200µg/ml	2.58	3.31	0.73	29	41	12
P. prostrata 300µg/ml	3.42	4.05	0.63	26	35	9
P. prostrata 400µg/ml	3.68	4.10	0.42	57	63	6
P. prostrata 500µg/ml	4.87	5.16	0.29	19	23	4

Table 5: Effect of *Ruellia prostrata* leaves on mitotic index of *Allium cepa* roots

Concentrations (µg/ml)	Dividing cells	Non dividing cells	Total number of cells	Mitotic index (%)
Group I (Water control)	897	95	1017	88.20
100	738	104	879	83.95
200	610	217	838	72.79
300	317	409	737	43.01
400	223	741	922	24.18
500	118	865	987	11.95

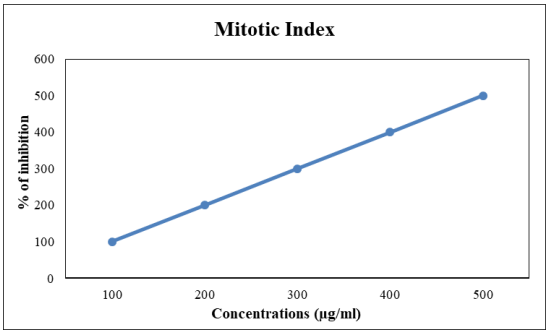

Figure 1: Effect of *Ruellia prostrata* leaves on mitotic index of *Allium cepa* roots

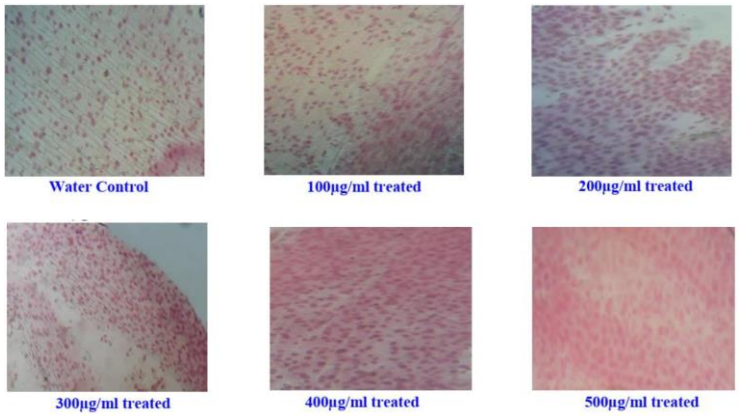

Plate 3: Effect of *Ruellia prostrata* leaves on mitotic cells of *Allium cepa* roots

Hence, the sterols from *Ruellia prostrata* leaves must be contributing to the anticancer potential of the herb. The result from the study showed that the extract of *Ruellia prostrata* leaves had excellent anti-mitotic activity that was comparable to the activity of methotrexate. Maximum numbers of non-dividing cells were observed. As a result of this cells arrest in mitosis and eventually die by apoptosis. Similar reports were observed in Shweta *et al*. (2013, 2014) and Thenmozhi *et al.*, (2011). Thus, it can assume the possible mechanism of the anticancer activity of *Ruellia prostrata* leaves may be due to the presence of flavonoids and phenolic compounds in the extracts. Our findings support the reported therapeutic use of this plant as a antimitotic or anticancer agent in the Indian system of medicine.

CONCLUSION

Overall, it concluded that above results suggest that the extract of *Ruellia prostrata* leaves has rich source of phytochemicals confirmed by qualitative and quantitatively. The biological activity evidenced by anti-mitotic activity assay proved by *Allium* assay. The potential use of *Ruellia prostrata* leaves as therapeutic agent holds great promise as the isolation of one or more cytotoxic chemicals from crude extract. The present investigation provides comprehensive *in vitro* that anti-mitotic demonstrates remarkable cytotoxic properties thus suggesting the feasibility of its possible promise as natural antitumor agent.

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