SHORT COMMUNICATION

Pharmacognostic and Pharmacological evaluation of *Ruellia tuberosa* L.

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Abstract: *Ruellia tuberosa* Linn. of family Acanthaceae was studied to investigate the microscopical, vein islet and vein termination numbers, palisade ratio, stomatal index and different chemical parameters. The antibacterial, antifungal and phytotoxic activities of the crude extract of the plant were also determined. Five bacterial species were used, of which, *Salmonella typhi, Escherichia coli*, and *Pseudomonas aeruginosa* were the most susceptible bacterial species to crude extract with MICs 10, 4.0 and 14mg/ml, respectively. Among the tested fungal species *Fusarium solani* and *Aspergillus niger* were more susceptible to crude extracts with MICs 1.34, 2.78 and 1.45µg/ml, respectively. At the concentration of 1000µg/ml the methnolic extract exhibited significant activity, at 100µg/ml the activity was good and at 10µg/ml the activity was moderate against *Lemna minor*. The above selected plants were shown by *in vitro* assays to be a potential source for natural antifungal, antibacterial and phytotoxic agents.

Keywords: *Ruellia tuberose,* Macro-microscopical study, chemical tests, antibacterial, antifungal, phytotoxic activities.

INTRODUCTION

Morphology and histology makes the first step to get knowledge about the diagnostic features, which are ascertained through the study of the tissue and their arrangement, cell wall and cell content (Youngken, 1950). Plant anatomy, the study of the internal structure of plants, has been a source of fascination and a field of scientific inquiry since the time of the earliest microscopists. The subject matter of plant anatomy centers on aspects of structure that can be observed with the light microscope. Much of our understanding of the cell-specific properties of specialized cell types such as sieve tube or tracheary elements is based on analyses using transmission and scanning electron microscopy and other tools of cell biology. Plant anatomists also aim to place what is known about the internal structure of plants in the broader context of the plant's external form, or morphology (Dengler, 2002). Not all the chemical compounds elaborated by plants are of equal interest to the pharmacognosist. Until relatively the so-called "active" principles were frequently alkaloids or specific glycosides usually with pronounced pharmacological properties; these received special attention, and in large measure constituted the principle plant drugs of the allopathic system of medicine. Other groups such as carbohydrates, fats, and proteins are of dietetic importance, and many such as starches and gums are used in pharmacy but lacked any marked pharmacological actions (Evans, 2002). Putiyanan *et al*. (2009) reported that the macroscopic characters were studied for sample collecting and microscopic characters of transverse section of Fak khaao's leaves were compared to the leaf powders showing the upper and lower epidermis, trichome, collenchyma, palisade mesophyll, spongy mesophyll, stoma (guard cell), vascular bundles, etc., which were similar to microscopic description of drug powders. The values of stomatal index, veinlet termination number, vein-islet number and palisade ratio were calculated for standardization of samples which were 11.84±1.77, 5.95±1.31, 2.38±0.40 and 4.49±0.73, respectively. Latha and Kannabiran (2006) reported the presence of tannins, saponins, flavanoides, phenolic compounds, cardiac glycosides and carbohydrates in *S. trilobatum. Ruellia tuberosa* is used in folk medicine due to its diuretic, diabetic, antipyretic, analgesic, and antihypertensive properties (Chiu & Chang, 1995). Recently, it is also being used as one of the components in a herbal drink in Taiwan (Chen *et al*. 2006). As no such literature was available on this plant therefore the present study was carried out.

MATERIALS AND METHODS

Fresh shoot material was collected from the Department of Botany, University of Peshawar. The plants were identified with the help of Flora of Pakistan by Prof. Dr. Abdur Rashid Plant Taxonomist (Ali and Qaiser, 2007). It was cleaned and washed and dried in air for 15 days and was used for different tests i.e micro chemical tests. The dried material was powdered and passed through mesh 60 and were preserved in airtight bottles to combat climatic conditions and moisture. Some fresh specimens were used to study anatomical parameters like vein islets numbers,

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vein termination number, palisade ratio, general anatomy of the root, stem and leaf and stomatal study. The anatomy of the root and stem was determined by following a standard method of (Puruis *et al*. 1966). For leaf anatomy Subrahmanyam, (1996) method was used. The vein islet numbers, vein termination number, palisade ratio was determined by following Evans, (2002). Various chemical parameters including alkaloids, mucilage, anthraquinon derivatives, calcium oxalate, tannin, lignin, starch, fats & oil, cutin, cellulose were determined by following Evans (2002). Protein content was determined by following Johnson (1940).

Preparation of extracts

Fifty grams of sample was separately soaked in 250ml 70% methanol for 72 hours. Thereafter, it was passed through Whatman filter paper No. 1823 thrice. The extract was concentrated in a rotatory evaporator at 40^{o}C and stored at 4^{o}C priors to use. The methanolic extract and the standard drug were dissolved in dimethylsulphoxide (DMSO) at the concentration of 2mg/ml and 1mg/ml for antibacterial, 24mg/ml and 1mg/ml for antifungal, 30mg/ml and 1mg/ml for phytotoxic activities, respectively.

Bioassay

Both gram negative and gram-positive bacteria including *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsella pneumoni* were tested. Nutrient agar medium was used for the growth of bacteria using agar diffusion method (Mariam *et al*., 1993). While nutrient broth medium was

Table 1: Anatomical features of the different parts of the *Ruellia tuberosa* L.

Plant cell	Value	Root		Stem	
		L (µm)	W (µm)	L (µm)	W (µm)
Epidermis	Minimum	22	10	34	27
	Maximum	38	19	55	39
	Mean	26	12	39	31
Xylem	Minimum	12	9	18	13
	Maximum	32	15	38	17
	Mean	22	11	27	14
Phloem	Minimum	34	20	39	29
	Maximum	56	28	55	41
	Mean	46	22	45	37
Endodermis	Minimum	18	9	22	18
	Maximum	34	15	34	23
	Mean	26	11	28	20
Pericycle	Minimum	14	10	16	12
	Maximum	26	16	27	20
	Mean	16	12	21	15
Pith	Minimum	10	7	-	-
	Maximum	15	11	-	-
	Mean	13	9	-	-
Cortex	Minimum	14	8	18	12
	Maximum	19	15	26	19
	Mean	16	12	23	15

Table 2: Microscopic Characteristics of the *Ruellia tuberosa* L.

S. No	Parameters	Values
1	Vein Islet Number	25.63-28.28
2	Vein Termination Number	55.21-58.43
3	Palisade Ratio	42.65-49.75
4	Stomatal Index (Upper surface)	14.23-15.42
5	Stomatal Index (Lower surface)	24.23-26.45

Table 3: Microchemical screening tests of the different parts of *Ruellia tuberosa* L.

Plant part	Alk	Muc	Anth	Cao	Sap	Tan	Sta	Fat	Pro	Lig	Cut	Cel
Root	+	-	-	+	-	+	+	-	+	-	-	+
Stem	+	+	-	+	-	+	+	-	+	-	-	+
Leaf	+	+	-	+	-	-	+	-	+	+	-	+
Flower	+	+	-	+	-	-	+	-	+	+	-	+

Table 4: Antibacterial activity of the methanolic extract of the whole plant of *Ruellia tuberosa* L.

S. No	Name of Fungus	Linear growth of sample (mm)	Linear growth of control (mm)	% Inhibition
1	*Salmonella typhi*	6	33	81.8
2	*Pseudomonas aeruginosa*	12		63.6
3	*Escherchia coli*	9		72.7
4	*Staphylococcus aereus*	17		48.4
5	*Klebsella pneumoni*	26		21.2

Table 5: Antifungal activity of the methanolic extract of the whole plant of *Ruellia tuberosa* L.

S. No	Name of Fungus	Linear growth of sample (mm)	Linear growth of control (mm)	% Inhibition
1	*Aspergillus niger*	7	45	84.4
2	*Aspergillus flavus*	35		22.2
3	*Fusarium solani*	15		66.6
4	*Candida albicans*	20		55.5
5	*Candida glabarata*	30		33.3

Table 6: Phytotoxic activity of the methanolic extract of the whole plant of *Ruellia tuberosa* L.

Plant name	Test plant	Conc. of compound (µg/ml)	% Growth regulation
Ruellia tuberosa	*Lemna minor*	1000	89.53
		100	67.25
		10	44.85

used for serial dilution method (Spooner & Sykes, 1972). Fungal tested species included *Fusarium solani*, *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Candida glabarata.* Sabouraud dextrose agar (SDA) was used as the growth medium for fungi using agar dilution method Mariam *et al*. (1993). Antibacterial, antifungal and phytotoxic activities of the plant were determined by following a method of Ahmad *et al*. (2009).

RESULTS

Epidermis of the root is rectangular in shape and is compactly packed. The mean length of the cells 26µ; mean width is 12µ. It is then followed by the cortical tissue. Mean length and width of the cell is 16µ and 12µ. Inner to the cortex is endodermis. Mean length and width are 26µ and 11µ. Single layer of pericycle is present, mean length and width of which is 16µ and 12µ. Xylem mean length and width are 22µ and 11µ. Mean length and width of phloem is 46µ and 22µ. In the center of the vascular bundle pith is present, mean length and width of which is 13µ and 9µ. Epidermis of the stem is spherical in shape and is compactly packed. The mean length of the cells 39µ; mean width is 31µ. It is then followed by the cortical tissue. Mean length and width of the cell is 23µ and 15µ. Inner to the cortex is endodermis. Mean length and width are 28µ and 20µ. Single layer of pericycle is present, mean length and width of which is 21µ and 15µ. Xylem mean length and width are 27µ and 14µ. Mean length and width of phloem is 45µ and 37µ. Epidermal cells of the leaf on adaxial surface are rectangular to hexagonal and polyhedral with smooth walls. The abaxial wall is irregular with undulating walls. Size of the epidermal cells; adaxial 133.75µ x 40µ. The abaxial one forming a network with the subsidiaries cells. Stomata is anisocytic type on abaxial side and staurocytic type on adaxial side. Size of stomatal comples; adaxial one, 148.5 µ x 59.5µ, abaxial one 90µ x 68.5µ, aperture size, adaxial one is 20.5µ (table 1).

Vein islet number ranges from 25.63-33.28, vein termination ranges from 55.21-58.43, palisade ratio ranges from 42.65-49.75, stomatal number of the upper surface of the leaf ranges from 14.23-15.42 and that of the lower surface ranges from 24.23-26.45 (table 2).

Alkaloid, Calcium oxalate, starch, protein and cellulose were present in all parts of the plant. Mucilage was absent from root and was present in other parts of the plant. Anthraquinon derivatives, saponins, fats and cutin were absent from all parts of the plant. Tannin was present in root and stem and was absent from leaf and flower. Lignin was absent from root and stem and was present in leaf and flower (table 3).

Methanolic extract of the plant exhibited low activity against *Klebsella pneumoni,* moderate activity against *Pseudomonas aeruginosa* and *Staphylococcus aereus* and significant activity against *Salmonella typhi* and *Escherchia coli* (table 4). The extract exhibited low activity against *Aspergillus flavus* and *Candida glabarata*, moderate activity against *Fusarium solani* and *Candida albicans* and significant activity against *Aspergillus niger* (table 4). At the concentration of 10µg/ml the methnolic extract exhibited significant activity, at 100µg/ml the activity was good and at 1000µg/ml the activity was moderate against *Lemna minor* (table 6).

DISCUSSION

Ferris *et al.* (2002) reported co-efficient of variance, stomatal density, stomatal index, epidermal cells area and number of epidermal cells per leaf. Kanwal *et al.* (2006) reported parenchyma cells, fibers, vessels, needle like elongated crystals and oil droplets in *Pongamia pinnata*. Khan *et al*. (2001) reported epidermal cells, collenchyma, tracheids and fibers in *Cyrtomium caryotideum*. Kumar *et al*. (2008) reported the vein islet number (13), vein termination number (18) and stomatal index (3.6) of the *Portulaca oleracea.* Abere *et al.* (2009) reported the palisade ratio, stomatal number and stomatal index of the upper and lower surfaces, vein islet number and vein termination number of *Dissotis rotundifolia*

Hadjiakhoondi *et al*. (2005) reported essential oils from three wild varieties of *Mentha longifolia*. Udayakumar *et al*. (2005) reported protein from *Tridax procumbens*. Ansari *et al*. (2007) reported saponins from *Balanites aegyptiaca*. Anke *et al*. (2006) reported new proanthocyanidins from *Rumex acetosa*. Different proanthocyanidins, a polymer fraction and the new phenolglycoside 1-O-β-D- (2, 4-Dihydroxy-6-methoxyphenyl)-6-O-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside were obtained.

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