



BOTANICAL AND BIOLOGICAL STUDIES OF *PLECTRANTHUS TENUIFLORUS* (VATKE) AGNEW. (LAMIACEAE) GROWING IN SAUDI ARABIA

NAHED M. WALY* AND SABAH H. EL GAYED**

***Department of Botany, Faculty of science, ** Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.**

ABSTRACT

The macro- and micro morphology of leaves and stem of *Plectranthus tenuiflorus* (Vatke) Agnew growing in Saudi Arabia were described and illustrated for their identification in both entire and powdered forms, in order to contribute for the medicinal plant identification. In Vitro antiprotozoal activities of methanol Extract against Malaria, Leishmaniasis, Sleeping Sickness and Chagas Disease were evaluated against *Plasmodium falciparum*, *Leishmania infantum*, *Trypanosoma brucei brucei* and *T. cruzi* respectively as well as toxicity against MRC-5 fibroblast cells. The result showed promising activity against *P. falciparum* and moderate, antiprotozoal activity against *T. cruzi* and *Leishmania infantum* and mild activity against *T. brucei*. In vitro antioxidant and free radical scavenging activity of the methanol extract was determined by spectrophotometric methods. The result indicate that the extract has a promising antioxidant activity and could be considered as potential source among the antioxidants natural origin.

Key words: Shar plant, glandular hairs; antimalarial; antitrypanosomal; antileishmanial; Cytotoxicity. Antioxidant and free radical scavenging

1. INTRODUCTION

The family Lamiaceae contains several genera, such as sage (*Salvia*), basil (*Ocimum*) and mint (*Mentha*), with a rich diversity of ethnobotanical uses. It has an important role as a source of medicinal and aromatic of commercial importance. Another important genus is *Plectranthus*, a large genus containing about 300 species found in Tropical Africa, Asia and Australia. Of the 300 species of *Plectranthus* 62 species were reported to be used as medicines, ornamentals, foods, flavors and fodder. (Catherine W. Lukhobaa, et al 2006). Several species of them are used as a folk medicine for skin irritations, antiseptics, vermicide and nausea (Narukawa et al., 2001). There are some reports (Sandra et al., 2001

and Abdel-Mogib et al., 2002) about the occurrence of unique diterpenoids in several *Plectranthus* species and moderate antibacterial activity.

Genus *Plectranthus* is represented in Saudi Arabia by six species: *P. arabicus*, *P. cylindraceus*, *P. tenuiflorus*, *P. barbatus*, *P. lanuginosus*, and *P. asirensis* (Chaudhary 2001). Mostly there are with more or less succulent leaves. They may be non-aromatic or slightly aromatic or with strong thyme like smell. They bear terminal spike like inflorescence or white or pale blue to deep blue flowers. All of these can be used as ornamental shrubs (Collonette 1998).

Some species of *Plecteranthus* are difficult to identify because of a lack of clear cut morphological criteria to discriminate not only among species within the genus but also among the closely related genera. This has resulted in numerous taxonomic problems in the naming of species with the result that species have often been placed in several closely related genera like *Coleus* (Paton et al. 2004).

Because of these taxonomic issues, different names have been used for the same species of *Plecteranthus* and thus it has been difficult to collate information about the ethnobotanical uses of this genus. Furthermore the most economy used medicinal species of *Plecteranthus* have a high degree of synonymy (Lukhoba, et.al. 2006). *Plecteranthus aegyptiacus* is the correct name of *Plecteranthus tenuiflorus* (Ryding &Paton 2001)

P. tenuiflorus (Shar) a well-known species in family Lamiaceae in Saudi Arabia ,it is an aromatic herb used in traditional medicinal plant in folk medicine as a non-specific treatment for ear ache and inflammation of middle ear (Albar et al., 2006), sore throat (Mossa et al., 2000; Rahman et al., 2004), respiratory system infections, and abdominal disorders (Abulfatih 1987).

Infections caused by protozoa such as *Trypanosoma*, *Plasmodium* and *Leishmania* are a major worldwide health problem causing significant morbidity and mortality in Africa, Asia and Latin-America. The incidence of these diseases has increased since the emergence of AIDS, while the difficulty of creating efficient vaccines underlines the continuous need for new, effective drugs that can replace or supplement those in current use because of their high toxicity and high cost. *Trypanosoma brucei brucei* causes sleeping sickness or human African trypanosomiasis (HAT) while *T. cruzi* causes Chagas disease in South America. HAT is a major cause of morbidity and mortality in sub-Saharan Africa and poses a major health and economic burden in these regions (Gross et al., 1999 and Remme et al., 2002) with an estimated 60 million people at risk of contracting this disease, which is fatal if left untreated (Barrett et al., 2003).

However, few data are available on their efficiency and safety, despite the fact that validation of traditional practices could lead to innovative strategies in protozoal disease control. What is more,

natural products from plants or other organisms represent a virtually inexhaustible reservoir of molecules, most of which is hardly explored and can constitute lead molecules for new drugs (Weniger et al.,2006).

The essential oil isolated by hydro distillation from leaves of *Plecteranthus tenuiflorus* was analyzer by GC and GC-MS and showed that thymol is the principle component of the essential oil (Al Baar et al 2010). (Smith et al., 1996) has reported that the principle component of the oil produced from plant harvested in Saudi Arabia was found to be thymol (85.3%).So our attention focused in this study of *Plecteranthus tenuiflorus* extract as antiparasitic and tested for some of the most dangerous protozoa as *Trypanosoma*, *Plasmodium* and *Leishmania*

The human body during oxidative stress produces large amounts of reactive oxygen species (ROS), these ROS may be dangerous because of their ability to attack numerous molecules, including proteins and lipids. In fact, it has been reported that ROS largely contribute to cellular aging mutagenesis and coronary heart disease through several ways, including membrane destabilization (DNA breakage). The cell can reduce the impact of ROS either by an endogenous system implicating enzymes or by an exogenous system using antioxidants, vitamin C and α -tocopherol (Djebbar Atmani et al., 2009). However, so far, there are no studies on the antioxidant potential of these plants. In this study, we examined the antioxidant capacity of extracts from these plants using reducing power and scavenging capacity against the radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The aim of the present study is:

- I. Studying the morphological and anatomical features of *Plecteranthus tenuiflorus*.
- II. Studying its antiparasitic activity for the treatment of protozoal diseases, such as malaria, leishmaniasis and trypanosomiasis.
- III. Studying its antioxidant activity.

2.MATERIAL AND METHODS

2.1.Plant material:

Plant was collected in two different seasons March, April 2008, December 2008 and January 2009, from the garden of King Abdul-Aziz University. Its identification was clarified by the staff member of Plant Taxonomy Department, College of Science King Abdul-Aziz University, Saudi Arabia, according to (Collonette 1998), after comparison with the herbarium samples at the department. Voucher specimens are deposited at the Herbarium of the Department of Natural Products, College of Pharmacy, King Abdul-Aziz University, Jeddah, Saudi Arabia.

2.2. Preparation of the crude plant extracts:

Fresh herb of *Plecteranthus tenuiflorus* of the two different seasons was cut into small pieces, dried in shade for fifteen days and finally in oven below 60. The dried plant material (1 kg) was ground into fine powder and exhaustively extracted with methanol. The extract was concentrated under vacuum at 40. The dark green viscous residue (65g) was kept away from light in a refrigerator at 4 °C. 50 mg of this extract was suspended in 10 ml of methanol and shaken automatically for 30min. then centrifuged. The clear supernatant solution was used to carry out the different experiments.

2.3. Morphological and Anatomical studies:

Morphological characters were determined from fresh materials collecting in different seasons. Taxonomic description of the plant was carried out according to (Migahid 1987), (Tackholm 1956) and (Chaudhary 2001) by comparison with reference herbarium specimen kept in King Abdul-Aziz herbarium.

Anatomical studies were conducted on fully flowered fresh plants. Portion of plants were stored in 70% ethanol. Developed middle cauline leaves, petiole and stems from fully flowered plants were used for anatomical study. Transverse sections of stem, leaves and petiole were made manually. Nikon binocular microscope was used for anatomical studies and photographic.

2.4. Chemicals and analytical instruments:

-Nikon binocular microscope was used for anatomical studies, Photographs were taken at

Olympus Bx51, Photomicroscope coupled to a DP72 exposure control unity

- Spectrophotometric measurement were carried out using Perkin-Elmer double-beam UV-Vis spectrophotometer Model Lambda 3B attached to a Panasonic KX-3626 printer and using 1 cm quartz cells.

Standard solution of ascorbic acid (provided by El-Amriya Pharm. Ind. (Alexandria- Egypt) was prepared as 1mg/ml in methanol, protected from light and kept in the refrigerator. 2,20-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Fluka (Buchs, Switzerland). Standard quercetin solution (purchased from Sigma) was prepared as 0.5 mg/ml in methanol, protected from light and kept in the refrigerator. Phosphomolibdic acid (Aldrich Chem. Co) (PMA) reagent was prepared as 10%w/vacuous solution.

2.5. Biological assays:

Whole-cell in vitro assays were performed according to procedures reported by (Vik et al. 2009) at the WHO-TDR supported screening center at the laboratory of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences of the University of Antwerp, Belgium using the standard protocols used in WHO-TDR Drug Discovery Network.

Reference drugs. For the different tests, appropriate reference drugs were used as positive control: vinblastine for MRC-5, chloroquine for *P. falciparum*, miltefosin for *L. infantum*, benznidazole for *T. cruzi* and suramin for *T. b. brucei*. All reference drugs were either obtained from the fine chemical supplier Sigma-Aldrich (Bornem, Belgium) or from WHO-TDR.

Test plate production. The experiments were performed in 96-well plates (Greiner, Bio-One, Wemmel, Belgium), each plate containing 16 samples at 4-fold dilutions in a dose-titration range of 64 µg/mL to 0.25 µg/mL. Dilutions were carried out by a programmable precision robotic station (BIOMEK 2000, Beckman, Brea, CA 92822, USA). Each plate also contained medium-controls (blanks: 0% growth), infected untreated controls (negative control: 100% growth)

and reference controls (positive control). All tests were run in duplicate.

2.5.1. Antiplasmodial activity.

The chloroquine-sensitive *P. falciparum* K1-strain was used to test plant extracts according to procedures reported by Vik et al. (2009). Percentage growth inhibition was calculated compared to the negative blanks.

2.5.2. Antitrypanosomal activity.

T. b. brucei: Trypomastigotes of *T. b. brucei* Squib-427 strain (suramin-sensitive) were cultured at 37°C and 5% CO₂ in Hirumi-9 medium (Hirumi and Hirumi, 1989), supplemented with 10% fetal calf serum (FCS). Assay was performed according to procedures reported by Vik et al. (2009) and Raz et al. (1997). *T. cruzi*: Tulahuen CL2 strain (nifurtimox-sensitive) is maintained on MRC-5 cells in minimal essential medium (MEM). The in vitro antitrypanosomal activity was determined according to procedures reported by Vik et al. (2009). The color reaction was read at 540 nm and absorbance values were expressed as a percentage of the blank controls.

2.5.3. Antileishmanial activity.

L. infantum amastigotes (MHOM/MA (BE)/67) were collected from an infected donor hamster and used to infect primary peritoneal mouse macrophages. The in vitro antileishmanial activity was determined according to procedures reported by Vik et al. (2009). Parasite burdens were determined microscopically after Giemsa staining and expressed as a percentage of the blank controls without sample.

2.5.6. Cytotoxicity assay.

MRC-5 cells were cultivated in MEM medium, supplemented with L-glutamine (20 mM), 16.5 mM NaHCO₃ and 5% FCS at 37°C and 5% CO₂. For the assay, 104 MRC-5 cells/well were seeded onto the test plates containing the pre-diluted samples and incubated at 37°C and 5% CO₂ for 72 h. Cells viability were determined fluorimetrically after addition of resazurin IC₅₀ was determined using a dose response curve and the results were presented in Table 4.

2.5.7. In Vitro Antioxidant activity: The antioxidant activity of methanol extract was determined by different in vitro methods such as, the DPPH free radical scavenging assay and Phosphomolibdic acid assay. All the assays were carried out in triplicate and average values were considered.

2.5.7.1. DPPH radical scavenging activity

The free radical scavenging activity of the methanolic extract of *Plecteranthus tenuiflorus* based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by (Adam et al. 2006). An aliquot (0.1 ml) of test sample (50, 100 or 200 µg/ml) was added to 3 ml of a 0.004% MeOH solution of DPPH and kept in dark. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0]/100$, where A₀ is the absorbance of the control, and A₁ is the absorbance of the extract/standard. The DPPH solution without sample solution was used as control. All tests were run in triplicate and averaged. Quercetin was used as positive control. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition percentage against extract concentration. The tests were carried out in triplicate.

2.5.7.2. Phosphomolibdic acid assay:

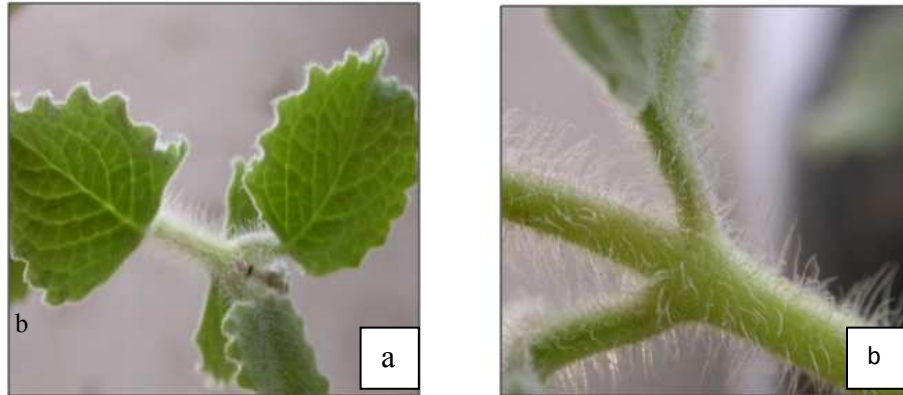
The reducing capacity of the extracts has been expressed as the ascorbic acid equivalents. The antioxidant power of the extracts has been assessed with the phosphomolybdenum reduction assay according to (Prieto et al 1999). The reagent solution contained ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (600 mM) mixed with the extract diluted in MeOH at the concentrations of 1, 5, 25, 50, 100, 200 and 500 µg/ml. The samples were incubated for 60 min at 37 °C and the absorbance of the green phosphomolybdenum complex was measured at 695 nm. For reference, the appropriate solutions of ascorbic acid have been used. The reducing capacity of the extracts has been expressed as the ascorbic acid equivalents

3.RESULTS

3.1. Morphological results:

Plecteranthus tenuiflorus plant is a downy, leafy herb, with stems about 60 cm long, semi prostrate, quadrangular succulent, branched and hairy. Leaves opposite decussate, petiolate, exstipulate ovate to elliptical 20 - 30 mm X 1.5 - 2 mm obtuse end crenate to dentate margin petiole 2-3 mm (fig1a,b)

and (fig3). Inflorescence erect spike 4 cm long, lilac-blue flowers 1.6cm long with a white upper lip. Flower: (fig. 2) Perianth distinct calyx and corolla. Calyx 5sepals bilabiate, persistent. Corolla 5 petals with bilabiate condition upper lip is four lobed and large shoe shapes, lower lips a single lobe. Androecia, 4 stamens 2 of these and style exerted beyond the lower lobe.



(Figure. 1a and b) *Plecteranthus tenuiflorus* in summer

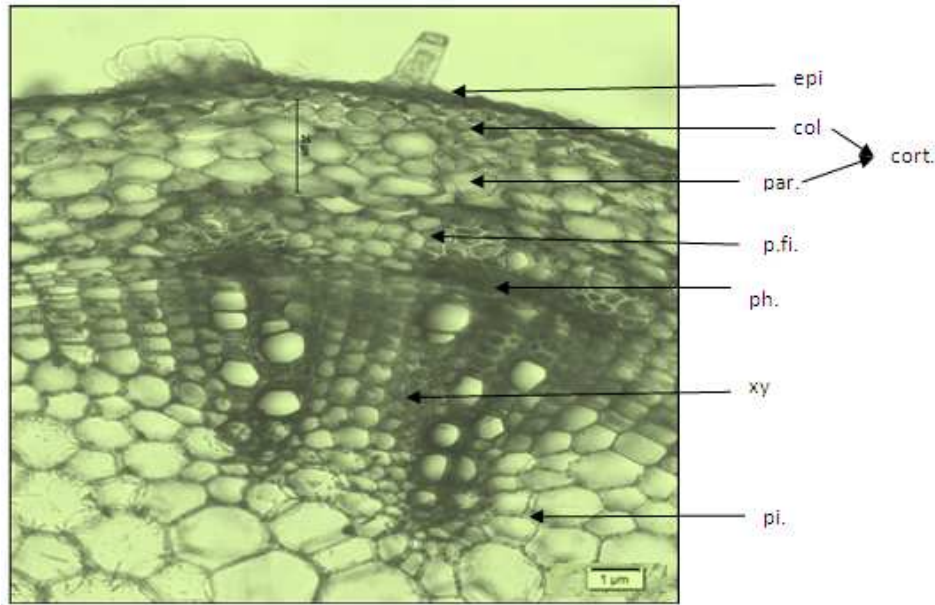


Summer flower: (fig. 2)

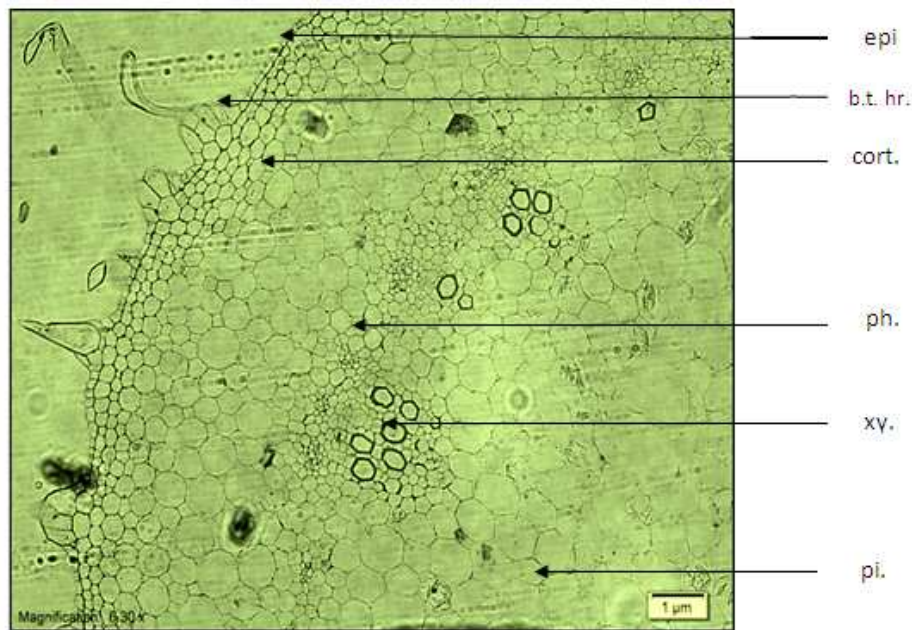


Plecteranthus tenuiflorus in winter (fig.3)

Plate 1: fig.1a & 1b *Plecteranthus tenuiflorus* in summer, fig.2. Summer flower, fig.3. *Plecteranthus tenuiflorus* in winter



T.S in summer stem (fig . 4)



T.S in winter stem (fig .5)

Plate 2:fig.4 &5. T.S. in stem in summer and winter, epidermis(epi), collenchyma (col), parenchyma(par), cortex(cort), pericyclic fiber(p.fi.), phloem(ph.), xylem(xy.), pith(pi.) base of tower hair(b.t. hr.)

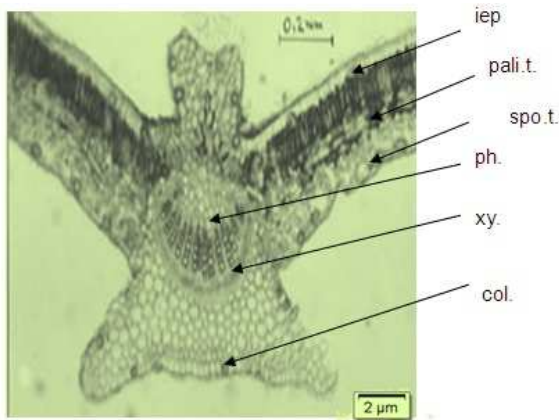
3.2. Anatomical results:

Leaf: Leaf blade: In transaction (fig. 6) , the single-layered epidermis oval in shape, covered with cuticle. There are glandular and eglandular trichomes similarly to the stem ones on both

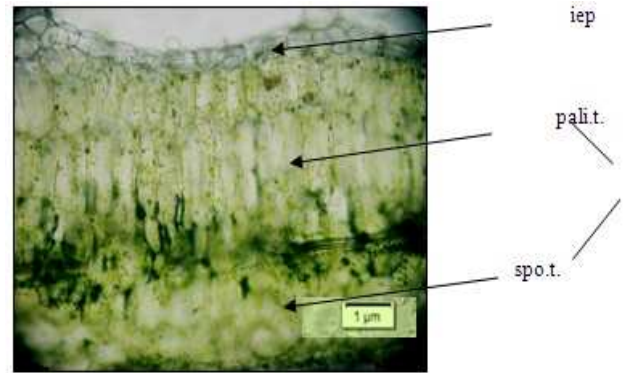
surfaces. Stomata type anomocytic and diacytic (fig. 10) with unequally sized subsidiary cells occur on the lamina, predominantly on the abaxial side and occur on both surfaces, located on the same level as epidermal cells Eglandular trichomes are 2-6-elongated cells (up to 80 μm) with striped cuticle

cuticular micro papillae. Glandular trichomes are generally of labiate and capitate types. Trichomes are located on both surfaces of the leaf. ...

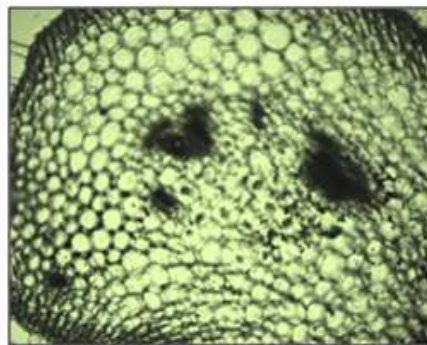
Complex hair base (Tower like hair) can be seen (fig.9)



T.S. in Leaf blade (fig. 6)



T.S. in mesophyll region: (fig. 7)



T.S in Petiole (fig. 8)

Plate 3: (fig. 6, 7, 8) T.S. in Leaf blade, epidermis (epi.), phloem (ph.), Xylem (xy.), collenchyma (col.) T.S. in Mesophyll, palisade tissue (pal.t.), spongy tissue (spo.t.), Mesophyll (Meso.). T.S. in petiole, vascular bundle (vas.b.)

The mesophyll (fig. 7) is dorsiventral and is differentiated into palisade and spongy parenchyma. The palisade tissue is under the upper epidermis. The shape of the palisade parenchyma in transverse section is cylindrical. The spongy parenchyma cells, circular or ovoid, are located under the palisade tissues.

The midrib region is well developed and forms a projecting part towards the outside. Vascular bundles are collateral. The xylem faces towards the upper surface, while the phloem faces the lower

epidermis. There are collenchymatous cells, 2-3-layered, under the upper and lower epidermis in the midrib.

Petiole: (fig. 8) It has nearly plain-convex contour in transverse section and a single-layered epidermis is covered with a thin and striate cuticle. There are glandular and eglandular trichomes similarly to the stem ones on both surfaces. The epidermal cells which surround the trichomes bases have slightly polygonal shape. A continuous strand of angular collenchyma, formed by one to two rows, encircles

the ground parenchyma, in which various vascular bundles are embedded. They are collateral and distributed as an open arc, where the two major bundles are seen in the centre.

Stem: The epidermis is composed of a single layer, barrel shaped, covered with thin cuticle. Eglandular 300- 280- 350 μ m and glandular hairs 100- 120- 150 μ m stalk and 25-35-47 μ m head are present (fig.9& 11) the former trichomes are unicellular or multicellular and uniseriate, consisting of about three to ten cells, with an acute apex and eventually a stalk cell dehydrated. These trichomes are coated with cuticle. Glandular hairs trichomes are numerous and show short unicellular with long multicellular stalk and uni- or bicellular head

Cortex: The collenchymas tissue located immediately under the epidermis, 2-3 layers layered irregular and fairly thick walled. Parenchyma tissue

is usually composed of squashed cells 4-5 layered (fig.4). The sclerenchyma tissue, located under parenchyma, as small separated bundles of fibers. Endodermis is indistinguishable The vascular cambia form xylem inward and phloem outward, being active mainly in the fascicular region and toward the xylem, although collateral bundles can be distinguished. The phloem is 3-4 layered and consist of irregular cells .The cambium is not observed. Secondary xylem form contiguous xylem groups of the bundles being separated from one another by interfascicular rays. Septate fiber with simple pits in isolated tissue (fig.13), vessels are polygonal with spiral thickening (fig.12), rays usually uniseriate. The pith consists of large hexagonal or polyhedral parenchymatous cells comparatively greater, forming small intercellular spaces

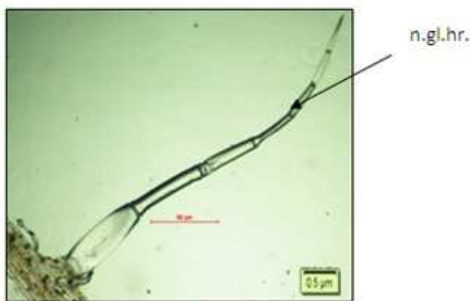


Fig (9)

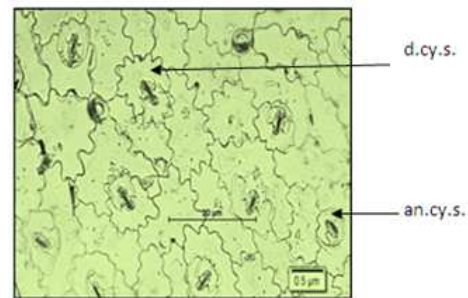


Fig (10)



Fig (11)

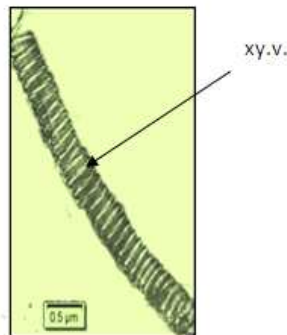


Fig (12)

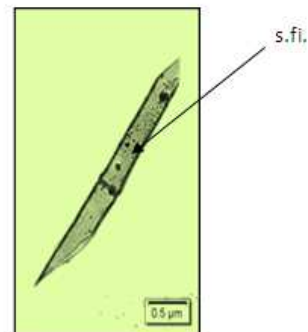


Fig (13)

Plate 4:fig.9-13.Non glandular hair (n.gl.hr.), glandular hair (gl.hr.), diacytic stomata(d.cy.s.), anomocytic stomata (an.cy.s.),xylem vessels (xy.v.), septate fiber(s.fi)

The comparative description of Morphological and Anatomical characters of *Plecteranthus*

tenuiflorus in seasonal variation is tabulated in Table 1

Table (1) Comparative Morphological and Anatomical characters of *Plecteranthus tenuiflorus* in seasonal variation

Characters	<i>Plecteranthus tenuiflorus</i> in summer	<i>Plecteranthus tenuiflorus</i> in winter
Morphology	Flowering stem up to 60 cm long	Flowering stem not exceed 40 cm long
	High dense cover of hairs	Low dense cover of hairs
	Flower extremely aromatic (fig.1a & b and fig.2)	No flower can be seen (fig.3)
Anatomy	Stem and leaf epidermis covered with thick cuticle	Stem and leaf epidermis covered with thin cuticle
	Eglandular and glandular trichomes in addition to Tower hairs can be seen in dense cover all over the plants	Eglandular bent trichomes on leaf and stem, rare glandular hairs
	Wide interfascicular region with distinguishable secondary growth in stem (fig 4.)	Collateral vascular bundle with limited growth in fascicular region (fig.5)

3.3. In vitro antiparasitic studies:

P. tenuiflorus extracts and essential oil has been not tested for their anti-parasitic activity although the chemical analysis of the essential oil of *Plecteranthus tenuiflorus* in the two different season was (85.3%) (Abdel-Mogib et al 2002) in Summer and (62.2%) in winter(Albar et al 2006) which promote the higher activity in the collected plant in Summer. In recent study there is significant and reproducible antischistosomal activity of *P. tenuiflorus* alcoholic extract against *Schistosoma*. The ethanol extract *P. tenuiflorus* showed the strongest activity against both *Schistosoma mansoni schistosomulae* and cercariae While, it was moderately active against *Schistosoma mansoni miracidia*. (A.Al- Badry et al 2011).

The extracts were tested in vitro for their potential antiprotozoal activity against *T. cruzi*, *T. b. brucei*,

L. infantum and *P. falciparum*, as well as against MRC-5 cell lines for cytotoxicity and evaluation of selectivity. To facilitate interpretation of the IC50-values in the different models, semi-quantitative activity scores were introduced (Table 2). Extracts showing score 3 against any of the protozoa with low cytotoxicity became subject for further fractionation and these fractions were re-evaluated in the model where the prime activity was found.

3.3.1. Antimalarial activity

The results in Table 2 revealed that plant extract exhibited a pronounced activity against *P. falciparum* K1 with IC50 10.08 µg/ml, Based on WHO guidelines and previous data (Jonville et al., 2008) antiplasmodial activity was classified as follows: high (IC50 <5_g/ml), promising (5 < IC50 <15_g/ml), moderate (15 < IC50 <50_g/ml) and

inactive ($IC_{50} > 50 \mu\text{g/ml}$). The tested methanolic extract showed promising activity (Table 2).

3.3.2. Antitrypanosomal activity

The result of plant extract exhibited moderate activity against *T. cruzi* ($IC_{50} 25.60 \mu\text{g/ml}$) and against *T. b. brucei* ($IC_{50} 47.26 \mu\text{g/ml}$). (Table 2).

3.3.3. Antileishmanial activity.

The result of plant extract exhibited moderate activity against *L. inf* ($IC_{50} 27.27 \mu\text{g/ml}$)

The results were expressed as the mean lethal dose 50 (LD_{50} = the drug concentration that reduced the number of viable cells by 50%) (Table 2).

Table (2) *In vitro* antiplasmodial, Antileishmania and Antitrypanosomal activities , cytotoxicity and selectivity index of the methanol extract.

	IC50 $\mu\text{g/ml}$	MRC-5	SI
Antiplasmodial activity Pf-K1	10.08 \pm 2	48.94 \pm 9.3	4.85
Antileishmanial activity <i>L. infantum</i>	27.27 \pm 7.2	48.94 \pm 9.3	1.79
Antitrypanosomal activity <i>T.cruz</i>	4.3 25.60 \pm	48.94 \pm 9.3	1.19
Antitrypanosomal activity <i>T.bruc</i>	47.26 \pm 5.6	48.94 \pm 9.3	1.03

(IC_{50} , $\mu\text{g/ml}$) = Inhibition concentration 50% \pm standard deviation. (LD_{50} , $\mu\text{g/ml}$) = Lethal dose 50% \pm standard deviation. *SI* Selectivity index = LD_{50}/IC_{50} .

A selectivity index (SI), corresponding to the ratio between the cytotoxic and antiparasitic activities of each plant extract, was calculated as follows:

$$SI \text{ Plasmodium} = \frac{LD_{50} - \text{MRC-5}}{IC_{50}}$$

3.3.4. Cytotoxicity

The methanol extract showed relatively high cytotoxicity, strongly suggesting that the observed activity against tested protozoa is due to non-specific effects.

3.4. *In vitro* antioxidant activity

3.4.1. DPPH radical scavenging activity

The DPPH antioxidant activity is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517nm and also for visible deep purple colour. When DPPH accepts an electron

donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The methanol extract of *Plecteranthus* exhibited a significant dose dependant inhibition of DPPH activity. The IC_{50} value was found to be $38.31 \mu\text{g/ml}$ quercetin per mg extract. Based on the previous results, the antioxidant activity of the methanolic extract was examined by exploring scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Table 3). The IC_{50} of all tested samples were determined. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. Quercetin. is commonly used as a reference standard.

Table (3) DPPH radical scavenging activity of the methanolic plant extract

	% Inhibition		
	50 µg/ml	100 µg/ml	200 µg/ml
<i>Plectranthus teniflorus</i>	24.1 ± 0.30	28.3 ± 0.13	31.6 ± 0.60
Quercetin	50.3 ± 2.20	64.6 ± 2.70	75.3 ± 3.10

3.4.2 Total antioxidant activity

The extracts exhibited dose dependent antioxidant activity (Table 4). The activity as measured by the intensity of the absorbance in tested concentrations

indicates that the methanolic extract exhibited obvious antioxidant activity. The IC₅₀ equal 0.544µg ascorbic acid equivalent per mg extract.

Table(4). The result of Phosphomolybdenum assay of the methanol extract of *P. teniflorus*

	% Inhibition		
	50 µg/ml	100 µg/ml	200 µg/ml
<i>Plectranthus teniflorus</i>	0.86 ± 0.01	0.95 ± 0.02	1.71 ± 0.05
Ascorbic acid	1.53 ± 0.06	1.87 ± 0.08	2.87 ± 0.09

4. Conclusion and Discussion

Morphological and Anatomical results have been compared between *Plecteranthus tenuiflorus* species in different seasons. Stem habit with dense hairs and appearance of red flowers are morphological characters differentiated between summer and winter specimens. Summer specimens were much longer and branched, with crowded leaves and dense cover of hairs all over the plants. Flowers extremely aromatic. Anatomical description of summer specimens in our study was different from winter specimens in some characters as shown in table 1. In summer specimens stem and leaf epidermis covered with thick cuticle. Eglandular and glandular hairs with unicellular and multicellular uniseriate stalk and uni – or bicellular head and complex base show dense cover all over the plant.

The present report of antiprotozoal activity of the methanolic extract will be a new challenge, as there are no reports in the literature on the antiprotozoal

activity. Therefore, testing of the isolated compounds of this plant species (summer species) is required and will be a subject of our further study.

Also studying other suspected antiprotozoal plants are needed to help in treatment of such dangerous diseases. The plant extract was less active than standard drugs used. This extract is composed of a large number of different compounds, and the active principles might show higher activity in their pure form which may indicate the possible isolation of more active compounds than the standard drug. Further detailed studies of the active plants for the isolation of major active constituents are in progress.

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