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Research article

Evaluation of pharmacognostic profile, phytochemical, and antimicrobial properties of *Psychotria vogeliana* Benth. (Rubiaceae), Leaf

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Abstract: The use of plants for the treatment of human diseases is as old as mankind on earth.. While there are reports on some *Psychotria* species there is virtually none on the plant *Psychotria vogeliana* Benth, (Rubiaceae). This research aims to evaluate the pharmacognostic, phytochemical and antimicrobial activities of the leaves of the plant. Macroscopic and microscopic characters of the plant material were evaluated and recorded. The plant material was sequentially extracted with n-hexane, ethyl acetate and methanol using a soxhlet extractor. The methanol extract was evaluated for acute toxicity using Lorke's method. Phytochemical analysis was carried out using standard methods while antimicrobial screening was done to test for antibacterial and antifungal activities following methods Clinical and Laboratory Standards Institute (CLSI) document M07-A9 2012). The phytochemical analysis was carried out using standard methods. Extraction yield**;** Methanol Extract = 9.73 %, ethyl acetate extract= 6.35 %, n-hexane extract = 7.22 %. The bitterness value (expressed as Mean ± SEM) was 0.65±0.04 IU/g, swelling index was insignificant. Results for Foaming Index indicated an insignificant foaming index. Ethanol soluble extractive (%) was 5.2±0.00, Water soluble extractive (%) was 5±0.012 Total ash (%) was 6.67±0.003, Moisture content (Loss-on-drying) (%) was 7.50±0.00. The leaf is hypostomatic with the paracytic type of stomata, with stomatal number 30.6 ±0.27, stomatal size 293.56 ±9.95 stomatal index 27.7 ±0.17%, vein islet number 3.82. The methanol extract was found to be non-toxic at doses below 400 mg kg^{-1}. The qualitative phytochemical analysis revealed the presence of alkaloids, reducing sugars, tannins, glycosides, saponins, terpenoids, steroids, flavonoids, phenols, cyanogenic compounds. The methanol extract has activity against all the tested organisms, but the n-hexane extract has no activity against *Pseudomonas aeruginosa,* while the ethyl acetate extract had no activity against *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli*. The methanol and n-hexane fractions showed activity against *C.albicans.* The qualitative phytochemical analysis revealed the presence of alkaloids, reducing sugars, tannins, glycosides, saponins, terpenoids, steroids, flavonoids, phenols, cyanogenic compounds. The plant *Psychotria vogeliana* Benth (Rubiaceae), leaf contains many major phytochemicals and methanol extract possesses antimicrobial activity. Further work should be carried out to investigate other activities of the plant.

Keywords: *Psychotria*, pharmacognostic, antimicrobial screening, phytochemistry.

INTRODUCTION

Plant-based systems continue to play an essential role in healthcare, and their use by different cultures has been extensively documented (Moerman, 1986). According to the World Health Organization (WHO) majority of people in Africa and Asia extensively use to address healthcare problems (WHO, 2002). Plant use as medicinal agents have transformed from use as crude drugs, refined materials, crude extracts and pure isolates from plants. Noteworthy plant-derived cures for human diseases include artemisinin (Klayman *et al.,* 1985) and artemisinin analogues from *Artemisia annua* L (Quinhaosu) (Klayman *et al.,* 1985; O'Neill & Posner, 2004), the anticancer vinca alkaloids, vinblastine and vincristine isolated from the Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don (Gueritte & Fahy, 2005; Roussi *et al.,* 2012), ephedrine, from *Ephedra sinica* Stapf (Ma Huang), the muscle relaxant, tubocurarine, isolated from genius *Chondrodendron* and genius *curarea* (Buss & Waigh, 1995). Paclitaxel isolated from the yew tree (*Taxus* spp.), Camptothecin from the happy tree, (*Camptotheca acuminata* Decne) and Podophyllotoxin from the May apple (*Podophyllum hexandrum* Royle and *Podophyllum peltatum* L). There are 415,180 unique names for plant-based medicines cited in one hundred and forty-three databases and publications. In 2017 the "Medicinal Plant Names Services'' collated more than 530,000 data records containing the scientific, pharmaceutical and common names used to refer to medicinal plants found in 143 sources. Since 1981, 1130 new therapeutic agents have been approved for use as

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pharmaceutical drugs, of which 593 are based on compounds from natural sources (Allkin, 2017). As at 2018, besides the national policies and regulations on Traditional and Complementary Medicine (T & CM) that had been developed in more and more Member States, the infrastructure on the governance of T&CM at national level had also been significantly improved (e.g. 107 Member States had a national office for TM and 75 Member States had a national research institute) (WHO, 2019). Successes achieved with natural and synthetic compounds being used as antimicrobial agents is under serious threat from the emergence of microorganisms resistant to their effect (Shaik *et al.*, 2014). Misuse, under-use and wrong choice of antimicrobial agents have led to the development of resistance by bacteria and other infectious microbial agents. Researchers have identified the expression of efflux pumps, prevention of binding to activity sites through binding site modification, and inactivation of the drug molecule as mechanism by which bacteria can resist antibiotics. Treatment failure due to bacterial resistance to existing antibacterial agents, as well as re-emergence of previously "eradicated" infections, and emergence of products of bacterial mutation, pose serious challenges to successful treatment of bacterial infections. This has necessitated the search for new effective antibiotic agents that are less liable to the development of resistance by bacteria. Investigating plants as a source of such agents is a good option.

Rubiaceae family is a large family of 630 genera and about 1300 species found worldwide, especially in tropical and warm regions. Many *Rubiaceae* family plants exhibited antimalarial, antimicrobial, antihypertension, antidiabetic, antioxidant, and anti-inflammatory activities. Bioactive compounds including indole alkaloids, terpenoids and anthraquinones have been isolated from these plants. (Sirigiri, 2015). Many members of the Rubiaceae family have been investigated for pharmacological activity, e.g. *Morinda lucida*, Benth, antimalaria (Olasehinde *et al.*, 2014), antidiabetic and antioxidant (Domekouo *et al.*, 2016), antinociceptive, anti-inflammatory and antipyretic (Nwobodo *et al.*, 2011), *Nauclea latifolia* Smith, antimalarial (Benoit-Vical *et al.*, 1998), *Nauclea diderichii* (De Wild) Merr, α-glucosidase inhibitors (Agnaniet *et al.*, 2016).

There is not much information on the plant *Psychotria vogeliana* Benth. (Rubiaceae) which is the subject of this research. This work aims to evaluate the plant's pharmacognostic and phytochemical, profile, and investigate its antimicrobial activities, thereby strengthening its ethnobotanical use in treating infections and contributing to man's capability in dealing with microbial infections.

MATERIALS AND METHODS

Description of the Plant [*Psychotria vogeliana* Benth (Rubiaceae)]

Figure 1. Habitat photograph of *Psychotria vogeliana* Benth.

Scientific Classification:

Group	Dicot
Order	Gentanales
Family	Rubiaceae
Sub-family	Ruboideae
Tribe	Psychotriaea
Genus	Pychotria
Species	*Pychotria vogeliana*

Synomyms:

Uragoga vogeliana (Benth) Kuntze (1891)
Psychotria pilifer (Hutch & Dlaziel (1931) excl. Talbot 234
Cephalis conuta (Hiern 1877)
Psychotri dusenti Standi (1931). (The Plant List, 2021)

Description: The plant is a shrub/sub-shrub with height ranging from 0ne to six meters with elliptical and/or obovate leaf blade. The leaf is glabrous and measures 6 to 25 cm in length and 2 to 12 cm wide with acuminate apex and cuneat to narrowly rounded base (IPNI, 2021).

Collection of plant samples

Collection of leaves *Psychotria vogeliana*, Benth was at Obukpa, Nsukka L. G. A. in Enugu State, South East Nigeria, between April and May 2017, and identified by Mr. Felix Nwafor, a taxonomist in the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. Herbarium specimens were deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. (Voucher number: PCG/UNN/0096). The names and family of the plant were crosschecked and confirmed through search on Medicinal Plant Services, (https://mpns.science.kew.org) and International Plant Names Index (http://www.ipni.org.)

Reagents and Equipment

Equipment: Shimadzu *ATX224* Analytical *Balance (*Shimadzu, Kyoto 604-8511, Japan), EIE –213EP Soxhlet extractor (EIE Instruments, Ahmedabad - 380006, Gujarat, India), Rotary Evaporator (RE-5003 50L, Henan Lanphan Industry Co., Ltd Zhengzhou, Henan, China), centrifuge (Eppendorf Centrifuge 5425 Eppendorf AG, Hamburg, Germany), Stuart Reciprocating Shaker (Model SSL2 by Stuart (Cole-Parmer, Staffordshire, UK). Spectrophotometer, (Jenway 6705 UV/Visible spectrophotometer ,Bibby scientific, UK), Manual single channel micropipette (Pipet-Lite XL Model, Mettler-Toledo Inc., Columbus, USA), Homogenizer (Frain Industries, Inc, IL, USA).

Reagents: Sodium nitroprusside, Naphthyl ethylene diamine dihydro chloride (NEDD), Sulphanilamide (Merck KGaA, Darmstadt, Germany), Phosphate buffer saline (PBS) pH.7.4, ferric chloride (Xilong Scientific Co., Ltd, China), Tween 80. Dragendorff's reagent, picric acid, Fehling's solutions A and B, rutin, Methanol, conc. Sulphuric acid concentrated ammonia (Merck KGa A, Darmstadt, Germany) gallic acid, potassium ferricyanide, NaOH, acetic anhydride, (Reagents, Charlotte, NC 28214, USA) ferric chloride (Xilong Scientific Co., Ltd, China) Na_2CO_3, Zouping Zhijin New Material Technology Co., Ltd Shandong, China)

Organisms used: The organisms were obtained from the Department of Pharmaceutical Microbiology of the University of Nigeria, Nsukka. Microorganisms used: The test microorganisms used (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus niger*).

Extraction: A 2-kg portion of the powdered leaf was sequentially extracted with n-Hexane, Ethyl acetate, and Methanol using Soxhlet extractor at 40^{o}C. The various extracts were concentrated using a rotary evaporator and stored in air-tight container and kept in fridge at 4^{o}C till needed.

Phytochemical analysis

Extraction: A 0.1 g of the extract was macerated with 80% methanol for 24 hours and filtered. The filtrated was used for the estimation of the different phytochemical. Quantitative and qualitative phytochemical analysis was carried out using standard procedures described by (Evans, 2009; Ghorai *et al.*, 2012; Harborne, 1973; Harborne, 1998; Sofowora, 2008) with slight modifications.

Physicochemical properties (Examination of the powdered leaf): Chemo-microscopic examination, fluorescence analysis, ashes and extractive values, were investigated according to the methods of (Evans, 2005; Mukherjee, 2002; Singh *et al.*, 2013; WHO, 2011)

Macroscopic and Microscopy

Macroscopic and Microscopic studies were carried out according to (Evans, 2009; Oduoye, 2013). Macroscopic evaluation of the collected leaves was performed through use of the eye and other sense organs. The colour, odour, taste, size and shape were recorded. Qualitative and quantitative parameters and leaf constants were determined using a light Olympus Tokyo (Japan No.271961) microscope at x40, x100 and x400 magnifications and photomicrographs were taken with a Motican Camera 2.0.

Acute toxicity studies

Acute toxicity was determined using the method of Lorke (1983).

Preparation of media

Nutrient agar and potatoe dextrose agar (PDA) media were prepared according to Clinical and Laboratory StandardsInstitute (CLSI) document M07-A9 2012).

Antimicrobial Activity Screening

The screening was done according to the method contained in (CLSI document M07-A9 2012). The Test microorganisms used: The test microorganisms used (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus niger*) were clinical isolates obtained from the department of pharmaceutical microbiology and biotechnology laboratory, university of Nigeria, Nsukka. Ciprofloxacin and fluconazole were used as standard (reference) antimicrobial agents.

Statistical analysis

All data were analysed using SPSS 20, with results in triplicates. Results were expressed as Mean ± SEM and $P \leq 0.05$ was considered significant. Missing data was handled by "Mean/Mode Substitution" approach, (Replace missing value with sample mean or mode and run analyses as if all complete cases).

RESULTS

Extraction yield

Methanol Extract	= 9.73 %
Ethyl Acetate Extract	= 6.35 %
n-HEXANE Extract	= 7.22 %

Physicochemical properties

The plant material had total ash 6.67%, acid-insoluble ash 1.25%, water-soluble ash 3.0%, sulphated ash 6.73%, Water-soluble extractive 5.0%, ethanol-soluble extractive 5.20% and loss-on-drying 7.50% (Table 1).

Table 1. Physicochemical analysis.

Total ash (%)	6.67±0.003
Acid-insoluble-ash (%)	1.25±0.003
Water-soluble ash (%)	3±0.032
Sulphated ash (%)	6.73±0.002
Water soluble extractive (%)	5±0.012
Ethanol soluble extractive (%)	5.2±0.00
Moisture content (Loss-on-drying) (%)	7.50±0.00

N = 3

Chemo-microscopy

The result of chemo-microscopy revealed the presence of cellulose, tannins, calcium oxalate, calcium carbonate, proteins, lignin and starch (Table 2).

Table 2. Fluorescence analysis.

Detection reagents	UV wavelength / Colour exhibited		
	Normal light	254 nm	365 nm
Petroleum ether	Army green	Cherry red	Purple
Methanol	Army green	Pink	Purple
50 % Hydrochloric acid	Army green	Brown	Black
50 % Sulphuric acid	Army green	Very bright green	Black
Ammonia	Yellow	Green	Purple
Ethyl acetate	Army green	Cherry red	Pink

Foaming Index

Results for Foaming Index indicated an insignificant foaming index as all dilutions of the plant drug gave no foam after shaking for 15 seconds.

Bitterness test

Bitterness value (expressed as Mean ± SEM) = 0.65±0.04 IU g^{-1}.

Swelling index

There was insignificant swelling of the leaf powder. Value (expressed as Mean ± SEM) = 2.60 ± 0.06 cm.

Table 3. Qualitative phytochemical analysis.

Tests	EXTRACT		
	Methanol Extract	Ethyl Acetate Extract	n-Hexane Extract
Alkaloids	+	+	-
Reducing sugars	+	+	+
Soluble carbohydrates	-	-	-
Tannins	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+

Steroids	+	+	+
Flavonoids	+	+	-
Cyanogenic glycosides	-	-	-
Phenols	+	+	+

Note: + indicates present, - indicated absent

Table 4. Quantitative phytochemical analysis.

	Phyto-constituents	**Extract**		
		Methanol	**Ethyl acetate**	**n-Hexane**
1	Tannins (mg /100 g)	13.19±0.58	23.66±0.02	6.57±0.08
2	Cyanogenic glycosides (mg / g)	NIL	NIL	NIL
3	Reducing Sugars (mg /100 g)	1144.93±113.89	886.90±87.9	405.82±59.33
4	Phenols (mg / 100 g)	2090.87±39.06	1793.55±13.43	526.03±30.76
5	Alkaloids (mg / 100 g)	72.97±2.92	35.27±1.46	32.0±0.75
6	Steriods (mg / g)	1.9±0.17	1.72±0.4	2.31±0.025
7	Glycosides (mg / 100 g)	72.7±0.74	53.88±1.61	43.42±0.46
8	Saponins (mg / g)	0.372±0.011	0.325± .022	0.11± 0.015
9	Flavonoids (mg / 100 g)	623.87± 47.33	786.35± 14.69	524.28±7.85
10	Terpenoids (mg / 100 g)	1648.86±72.87	804.17±33.24	545.112±28.61

Table 5. Antibacterial activity screening.

Organisms	MIC (μg ml^{-1})			
	Methanol extract	Ethyl Acetate extract	n-Hexane extract	Std. (Ciprofloxacin)
S*taphylococcus aureus*	400	R*	1000	7
Escherichia coli	600	R*	1000	10
Bacillus subtilis	300	700	800	6
Klebsiella pneumonia	700	1300	1400	6
Pseudomonas aeruginosa	1300	R*	R*	11
Salmonella typhi,	800	1500	1800	8

Note: *R implies resistance (organism not sensitive at doses of 20 μg ml^{-1})

Table 6. Antifungal activity screening.

Organisms	MIC (μg ml^{-1})			
	Methanol extract	Ethyl Acetate extract	n-Hexane extract	Std. (Fluconazole)μg/ml)
Candida albicans,	1100	R	900	5
Aspergillus niger	R	R	R	8

Note: *R implies resistance (organism not sensitive at doses of 20 μg ml^{-1})

Acute toxicity

There was 100% survival in the group dosed with 100 mg kg^{-1} and 100 % death in the 1600 mg kg^{-1} group. The LD_{50} was calculated as the geometric mean of these two dose regimens. *Psychotria vogeliana* Benth leaf methanol extract was found to be non-toxic at doses below 400 mg kg^{-1}.

Macroscopy

The leaf is elliptic with acuminate apex, acute base and entire margin. It is deep green on the upper surface and pale green on the lower surface. It measures 12-15 cm in length and 6-10 cm in width.

Leaf microscopy

The plant is hypostomatic with the paracytic type of stomata, polygonal cells and straight anticlinal cell walls. It has Unglandular, uniseriate, multicellular trichomes, Stomata number 30.60±0.27, Stomata index 27.7±0.17, Stomata size 293.86±9.95mm^2, Vein islet number 3.82 mm^{-2} (Table 7).

Table 7. Foliar epidermal study of *Psychotria vogeliana* Benth.

Epidermal cell	Epidermal cells are polygonal in shape with straight anticlinal cell walls
Stomata type	The leaf is hypostomatic with the paracytic type of stomata
Trichome type	Unglandular, uniseriate, multicellular. Glandular trichomes are absent.
Stomata number	30.60±0.27
Stomata density	180±1.57 mm^{-2}
Stomata index	27.7±0.17
Stomata length	19.70±0.45mm
Stomata width	14.91±0.32mm
Stomata size	293.86±9.95mm^2
Trichome density	18.47 mm^{-2}
Trichome length	155.06±31.61mm
Vein islet number	3.82 mm^{-2}
Veinlet termination number	3.50 mm^{-2}

Note: N = 10

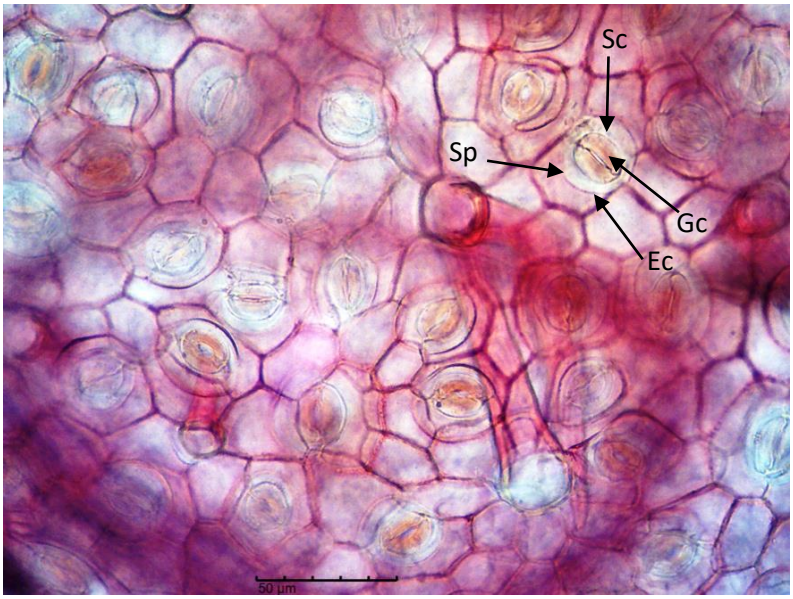

Figure 2. Abaxial surface showing paracytic stomata **x400 [**Sp = stomatal pore; Sc = subsidiary cell; Gc = guard cell; Ec = epidermal cell]

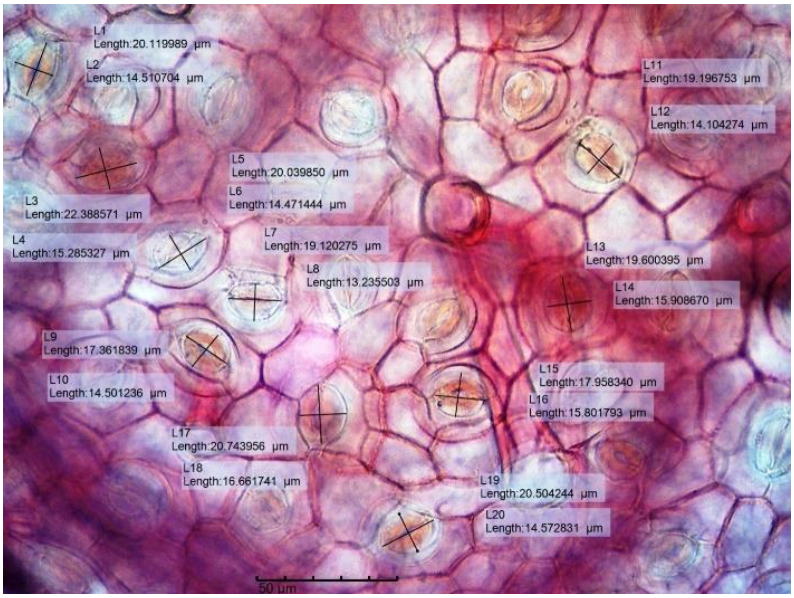

Figure 3. showing stomatal dimensions (Mag x 400)

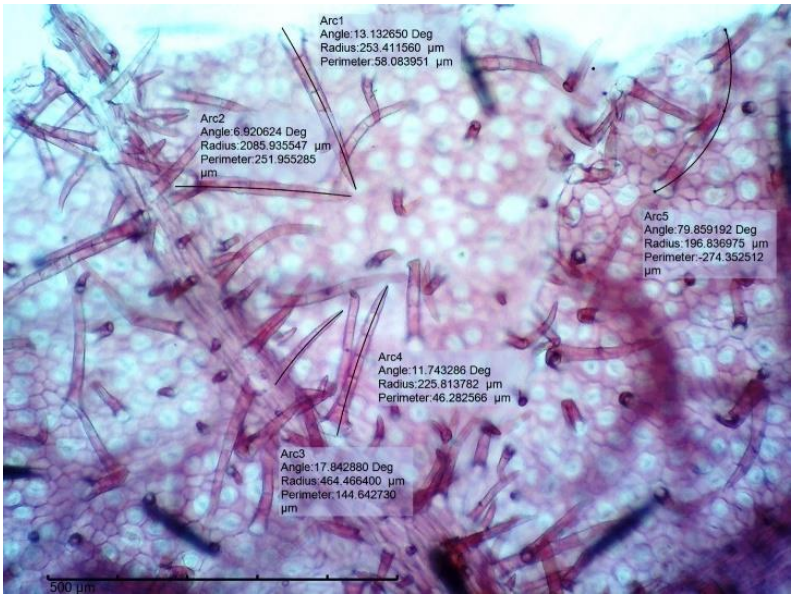

Figure 4. Showing uniseriate multi-cellular trichomes (Mag x 100)

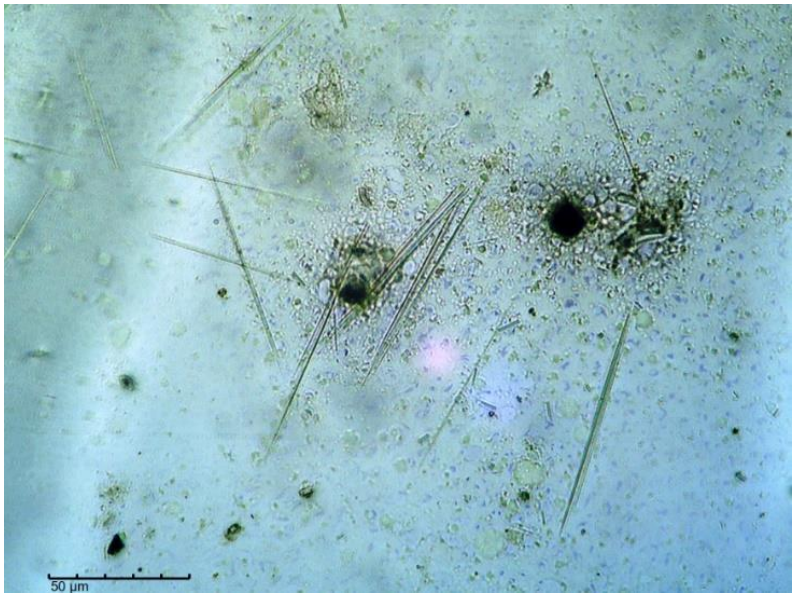

Figure 5. Showing raphides. (Mag x 400)

DISCUSSION

Traditional/herbal medicines have proven to be effective in the treatment and/or management of human healthcare problems. Healthcare provision involving herbal medicines, traditional treatment and traditional practitioners is still very common among many human societies (Chan, 2013). Situations are increasingly arising where bacteria that are resistant to most, or even all, available antibacterial drugs are causing serious infections that were readily treatable until recently. This means that progress in modern medicine, which relies on the availability of effective antibacterial drugs, is now at risk (WHO, 2014). Investigation into the antimicrobial activity of this little-documented plant (*Psychotria vogeliana* Benth) is timely, and if taken further may be of great benefit. The hypostomatic nature (stomata only occur on the lower surface) and paracytic type of stomata are characteristic. These features are of value in the identification, characterization, and quality control of *Psychotria vogeliana* Benth leaves. The identified paracytic stomata type is in agreement with the observation of Obembe (2015).

The virtual absence of swelling or foaming will be a useful guide in preparation of aqueous formulations or design of researches involving shaking of prolonged soaking. The phytochemical analysis of *Psychotria vogeliana* Benth, (Rubiaceae), leaf in this research revealed the presence of alkaloids, reducing sugar, tannins, glycosides, saponins, terpenoids, steroids, flavonoids, phenolics. The findings of this research are in line with results on other members of the species. The genus *Psychotria* is an abundant source of alkaloids as well as flavonoids, terpenoids, tannins (Iniyavan *et al.*, 2012; Lu *et al.*, 2014; Phan *et al.*, 2007). This is reflected in table 4.

The methanol extract has activity against all the tested organisms, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi* respectively. The activity of the methanol extracts is in line with other observations. According to results recorded with the *Rubiaceae* species, other researchers found the greatest activity in ethanol extracts, showing the strong capacity of his solvent to extract the antibacterial compounds of the Rubiacea (Karou *et al.*, 2011). *Psychotria vogeliana* Benth, (Rubiaceae), leaf contains many major phytochemicals and has antimicrobial activity. This result is in line with the results of earlier cited works. The methanol extract possesses the highest antibacterial activity.

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