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Antimicrobial Activities of Plant Extracts against *Streptococcus pneumoniae* Isolated from Pediatric Patients at Federal Teaching Hospital Abakaliki, Ebonyi State

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Abstract

Antibiotic-resistant genes have become a threat to synthetic or conventional medications and because of this much work has been done on using plants and plants part to treat disease caused by bacteria, Herbal medicine has served as effective treatment against various diseases caused by pathogenic bacteria and multi drug-resistant strains of bacteria which made it advantageous over synthetic medications. This study aimed to reveal the sensitivity of *Streptococcus pneumoniae* from clinical isolate and perform antibacterial assay on the organism using plants leaf extracts of *Ocimum gratissimum*, *Sida acuta*, *Newbouldia laevia* and *Mimosa pudica.* Gram staining and various biochemical test were used for the identification of *Streptococcus pneumoniae.* The plants leaves were aseptically washed, dried and ground into fine powder and diluted in varying concentration and agar well diffusion method was used to test for the antimicrobial properties of this plants on *Streptococcus pneumoniae* at various concentrations as follows 0.1 g/ml, 0.4 g/ml, 0.6 g/ml and 1 g/ml. The plants extract of *Ocimum gratissimum* showed a greater antibacterial effects on *Streptococcus pneumoniae* in high concentration more than other plant extracts while *Sida acuta* and *Newbouldia laevia* plant extract

showed weak antibacterial properties to the organism. This proves that *Ocimum gratissimum* and *Mimosa pudica* leaves have good and strong antibacterial properties against *Streptococcus pneumoniae* than *Sida acuta* and *Newbouldia laevia* and can be used as antibacterial agent at adequate concentrations.

Keywords

Conventional, Herbal, Antibacterial, Extracts, Concentrations

1. Introduction

Decades ago, natural products with antimicrobial effect were investigated in order to eliminate and minimize the use of synthetic antibiotics which micro-organisms are able to develop resistant genes to and has detrimental side effects to human health. The emergence of resistant strains of microbes against most chemotherapeutic agent has resulted in the use of plants parts and plants extract which are much safer to the human body than its synthetic counterparts [1].

Sida acuta is one of the plants currently used by indigenous people for the management of some health problems. This plant is an erect, branched small perennial herb or small shrub of about 1.5 m height [2]. All parts of this tree, including leaves, bark, root, seeds and flower are used in folkloric medicine. The plant is native to Mexico and Central America but has spread throughout the tropics and subtropics [3]. In traditional medicine, the plant is often assumed to treat diseases such as fever, headache, skin diseases, diarrhea, and dysentery. *Sida acuta* has been scientifically studied for its numerous pharmacological profiles such as: antioxidant, antimicrobial and antibacterial, antimalarial, antiulcer, anti-inflammatory, antipyretic, hepatoprotective. AIl parts of the plant are used for therapeutic purposes, but the leaves are the most frequently used. Leaves are considered to possess demulcent, diuretic, anthelmintic and wound-healing properties, and are used to treat rheumatic affections [4]. The leaves decoction is used to treat abdominal pain, hemorrhoids, azoospermia and oligospermia [5]. The leaf juice is also used in India for vomiting and gastric disorders [6]. The roots of the Sida species are considered excellent adaptogenic and immunomodulator, general nutritive tonic and prolonged life; useful in tuberculosis and in diseases associated with injury, heart diseases, cough and respiratory diseases [7]. Root is also claimed to possess aphrodisiac, antirheumatic, stomachic, diaphoretic, diuretic, antipyretic and wound healing properties [8]. The root extract is taken in the case of leucorrhoea [9], breathing problems and cough [10]. In Papua New Guinea, the fresh root is chewed for the treatment of dysentery [11]. In Indian traditional medicine, the root of *Sida acuta* is extensively used as a stomachic, diaphoretic and antipyretic.

Ocimum gratissimum is one of the medicinal herbs in Nigeria used in treatment of some infectious disease, the Ocimum oil is active against several species of bacterial and fungi, for example shigella, salmonella, proteus, Trichophyton rubrum

etc [12]. *Ocimum gratissimum* is rich in alkaloids, tannins, phylates flavonoids and Oligosaccharides and it has tolerable cyanogenic glycoside content [13] which is the chemical compound active against microorganisms. The described pharmacological properties of the plants involve the ant-plasmodial, antimicrobial, antioxidant, and many other properties. Some studies resulted in the isolation of single compounds while the others just demonstrated the activity of the crude extracts. *Ocimum gratissimum* have been asserted to provide various culinary and medicinal properties. These medicinal properties exact bacteriostatic and bactericidal effects on some bacteria.

Mimosa pudica is a neglected weed that has been studied for its numerous ethnobotanical uses. It is one of the sought-after plants for its pharmacological properties, which include antidiabetic, antitoxin, antihepatotoxic, antioxidant, and wound-healing properties [14]. The plant has been widely mentioned in Ayurveda and the Unani medicine system. In the past, and still in several parts of the world, the different parts of Mimosa are used to relieve several illnesses and health discomfort. The root decoction of this plant is used to relieve toothache. *Mimosa pudica* is reported to stop the bleeding and speed up the healing of the wound. It is mostly utilized in herbal remedies for gynecological conditions [15]. The phytochemicals of plants are attributed to multiple pharmacological activities, and they can be screened with some in vitro assays, believing that they have the same in vivo potency. The presence of different phytochemicals, such as carbohydrates, terpenoids, phenols, aliphatic, and aromatic molecules, and peptides are responsible for the antimicrobial activity of medicinal plants [16].

Newbouldia laevia is a valued herb in tropical African medicine, and as such was used and grown for centuries in Togo (West Africa). From the six most used plant extracts to treat life-threatening disease malaria in Togo, *N. laevia* was reported in a study to be the most active [17]. *N. laevia* is frequently cited to treat several diseases, including diarrhea, dysentery, and some sexually transmitted diseases or used as anthelmintic [18]. Medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. Recently, [19] reported ten compounds isolated from the methanolic root bark extract of *N. laevia* that demonstrated strong antimicrobial activity against twenty-one microorganism's bacterial species as well as three yeasts [20].

One of the most common Gram-positive pathogens that causes pulmonary inflammations is *Streptococcus pneumoniae*, which is known as pneumococcal in medical microbiology. It was recognized as a major cause of pneumonia diseases in the late 19th century causing many types of pneumococcal infections in children and the elderly other than pneumonia in many communities acquired respiratory infections [21]. Streptococcus pneumonia is a major source of morbidity and mortality worldwide. It is estimated that about 1 million children die of pneumococcal disease every year in WHO report. Pneumococcal infections are the leading cause of death from a vaccine-preventable illness in children aged less than 5 years [22]. Invasive diseases caused by pneumococci include meningitis, sepsis

and pneumonia, [23]. Risk factors for invasive pneumococcal disease (IPD) include age (with incidence being highest in young children aged less than 2 years and the elderly aged over 65 years), ethnicity, geographic location, concomitant chronic illnesses and attendance in daycare centers [24].

Streptococcus pneumoniae is the leading cause of invasive diseases such as pneumoniae, meningitis and sepsis. Furthermore, the emergency of multidrug-resistant *Streptococcus pneumoniae* has been focused worldwide as it is one of the most important bacterial pathogens that affect respiratory system [25].

2. Materials And Methods

2.1. Study Area

This study was carried out in Abakaliki capital city of Ebonyi State in South-Eastern Nigeria, located between the coordinate's latitude 5˚40 and 6˚54N and longitude 7˚30 and 8˚46E [26]. Ebonyi state is physically bounded to the east by Cross River State, to the north by Benue State, to the west by Enugu State and to the south by Abia State. The vegetation is a mixture of eastern prototypes comprising of semi-savannah grassland with forests and swamps, population (2022 estimated) is 4,816,675. Their main livelihood is agriculture and the major seasons are the rainy and dry season. The wet season is warm, oppressive and overcast and the dry season is hot, muggy and partly cloudy. Over the course of the year, the temperature typically varies from 65˚C to 89˚C and is rarely below 58˚C or above 92˚C.

2.2. Sample Collection

Clinical isolates used were collected from a pediatric patient at Federal Teaching Hospital Abakaliki.

2.3. Collection and Identification of Plant Material

Four plant Species (*Mimosa pudica*, *Ocimum gratissimum*, *Sida acuta* and *Newbouldia laevia*) were collected from different areas in Ikwo Local Government Area, Ebonyi State, Nigeria. The plants were identified by a Botanist at Alex Ekwueme Federal University Herbarium. For antimicrobial activity, plant materials were washed, air-dried, grinded into powder and stored at room temperature until use.

2.4. Isolation of Pure Culture

A loop full of the isolate was collected from a mixed culture and inoculated on a chocolate media using a sterile wire loop and incubated at 37˚C for 24 hr. The plate was evaluated on the basis of colony morphology (colour, consistence and texture). The pure culture sample was taken through a gram-staining process. Those that appeared gram-positive were placed on a shortlist for final definitive evaluation. Sample on the shortlist was subcultured on blood agar after 24 hours.

All media were prepared according to the manufacturer's instructions

2.5. Preparation of Plants Ethanol Leave Extract

Four grams of each plant powder were soaked in 20 ml of 70% Ethanol for 24 hrs with interval shaking at 37˚C. The mixtures were centrifuged at 5000 rmp for 5 minutes and the supernatant were frozen at 20˚C and evaporated by freeze-drying. The extracted powders were stored at 20˚C.

2.6. Inoculum Preparation

The disc diffusion method was performed in reference to the available CLSI procedures. This was done so as to assess the antibacterial activity of the plant extracts. *Streptococcus pneumoniae* bacterial culture that will be adjusted to 0.5 McFarland standard was used to lawn 6 mm Muller Hinton agar plates evenly. A sterile wire loop was used. In order to avoid loss of viability, the bacterial suspension was used within 15 minutes of standardization. The plates were dried by leaving their lids ajar for 15 minutes and then used for the sensitivity test.

2.7. Antibacterial Activity of the Plants Extracts

The *in-vitro* antibacterial activities of the plant extracts were evaluated using clinical isolate of gram-positive *Streptococcus pneumoniae.* The plants' extracts were serially diluted from 1, 0.6, 0.4, and 0.1 and were inoculated with I microliter of cfu/ml of *Streptococcus pneumoniae.* The inoculated plate was incubated at 37˚C for 24 hrs after which the zone of inhibition was observed, measured with meter ruler and recorded.

3. Results

3.1. Isolation and Identification of *Streptococcus pneumoniae*

The clinical isolates were subcultured into a chocolate agar and incubated for 24 hrs at 37˚C. After 24 hrs the pure cultures were identified by various biochemical tests, gram staining and their colony characteristics and microscopy. Biochemical tests like sugar fermentation test (glucose and lactose test), catalase test, oxidase test, VP test, Indole test, and Bile test where carried out and the result showed a positive result on bile, glucose and lactose test. And a negative result on oxidase test, Voges, Proskauer test, catalase and indole test. The organism appeared purple after the gram stain test, indicating the organism is gram-positive.

3.2. Antibacterial Activity of the Plants Extracts

The *in-vitro* antibacterial activities of the plant extracts were evaluated using clinical isolate of gram-positive *Streptococcus pneumoniae.* The plants extracts were serially diluted from 1, 0.6, 0.4, 0.1 and were inoculated with I microliter of cfu/ml of *Streptococcus pneumoniae.* The inoculated plates were incubated at 37˚C for 24 hrs after which the zone of inhibition was observed, measured with meter ruler and recorded.

3.3. Isolation and Identification of *Streptococcus pneumoniae*

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Table 1. The result of colonial characteristics, microscopy and biochemistry characterization.

Colonial Characteristics	Microscopy	Biochemical Characteristics								Suspected Organism
		Gram staining	Catalase	Oxidase	Voges Proskauer	Indole	Bile Solubility	Lactose	Glucose	
Flat raised edges with greenish discolorations	Round in short chains	+	-	-	-	-	+	+	+	*Streptococcus pneumoniae*
Dome-shaped colony with greenish colonies	Round in long chains	+	-	-	-	-	+	+	+	*Streptococcus pneumoniae*
Green raised edges flat colonies	Round in short chains	+	-	-	-	-	+	+	+	*Streptococcus pneumoniae*

3.4. Preparation of Plant Extract

The plant leaves were aseptically collected and washed and dried under room temperature before grinding using an electric grinder, 4 gm of each plant powder were soaked in 20 ml of 70% Ethanol for 24 hrs with interval shaking at 37˚C. The mixtures were centrifuged at 5000 rmp for 5 minutes, and the supernatant was frozen at 20˚C and evaporated by freeze-drying and then diluted at 1 g/ml, 0.6 g/ml, 0.4 g/ml, 0.1 g/ml concentration.

3.5. Antimicrobial Activities of the Plant Extract

Antimicrobial activity of the plant extracts was examined using agar well method, the prepared inoculum was inoculated in Mueller hinton agar and 4 wells were made using the cork borer before the addition of varying concentration of ethanol plant extract and incubated at 37˚C for 24 hrs and the zone of inhibition was observed, measured and recorded as shown in Table 2.

Table 2. The Result of Inhibition zone diameter of antimicrobial activity of *Ocimum gratissimum* against *Streptococcus pneumonia.*

Tested Organisms	Extract Concentration g/ml			
	0.1	0.4	0.6	1.0
Streptococcus pneumoniae 1	NIL	NIL	8 mm	10 mm
Streptococcus pneumoniae 2	NIL	8 mm	8 mm	10 mm
Streptococcus pneumoniae 3	10 mm	11 mm	14 mm	15 mm

Ocimium gratissimium, *Mimosa pudica*, *Sida acuta*, *Newbouldia laevi* plants extract were tested on *Streptococcus pneumonia* isolate using agar well diffusion method and incubated at a temperature of 37˚C for 24 hours and the zone of inhibitions were measured using a metre rule, *Ocimum gratissimum* showed a better zone of inhibition than other plant extracts at 15 mm of 1 g/ml on SP3 as shown in Table 2 while *Mimosa pudica* showed a better zone of inhibition of 13 mm at 1 g/ml concentration on SP2 as shown in Table 3,

Table 3. The Result of Inhibition zone diameter of antimicrobial activity of *Mimosa pudica* ethanol extract against *Streptococcus pneumoniae* species.

Tested Organisms	Extract Concentration g/ml			
	0.1	0.4	0.6	1.0
Streptococcus pneumoniae 1	NIL	NIL	NIL	NIL
Streptococcus pneumoniae 2	NIL	NIL	9 mm	13 mm
Streptococcus pneumoniae 3	NIL	8 mm	9 mm	10 mm

Whereas *Sida acuta* and *Newbouldia laevi* gave a zone of inhibition of 12 mm on SP3 and 10 mm on SP2 respectively as shown in Table 4 and Table 5 respectively.

Table 4. The Result of Inhibition zone diameter of antimicrobial activity of *Sida acuta* ethanol extract against *Streptococcus pneumoniae*.

Tested Organisms	Extract Concentration g/ml			
	0.1	0.4	0.6	1.0
Streptococcus pneumoniae 1	NIL	NIL	8 mm	10 mm
Streptococcus pneumoniae 2	NIL	NIL	NIL	NIL
Streptococcus pneumoniae 3	NIL	NIL	10 mm	12 mm

Table 5. The Result of the Inhibition zone diameter of antimicrobial activity of *Newbouldia laevia* ethanol extract against *Streptococcus pneumoniae*.

Tested Organisms	Extract Concentration g/ml			
	0.1	0.4	0.6	1.0
Streptococcus pneumoniae 1	NIL	NIL	NIL	NIL
Streptococcus pneumoniae 2	NIL	NIL	11 mm	10 mm
Streptococcus pneumoniae 3	NIL	NIL	8 mm	9 mm

4. Discussion

Plant leaves have been used overtime as alternative to conventional medicines against bacterial infections and gastroenteritis. Plants parts with antimicrobial properties have been investigated in order to eliminate and minimize the use of synthetic antibiotics which organisms develop resistant to easily [27].

The aim of this research was to ascertain the antibacterial activities of *Osimum gratissismum*, *Sida acuta*, *Newbouldia laevia* and *Mimosapudica* plant extract on *Streptococcus pneumonia.* Agar well diffusion method was used. Findings from this research revealed that *Ocimum graticismum* and *Mimosa pudica* plant extract have higher and better antimicrobial activity than *Sida acuta* and *Newbouldia laevi* on *Streptococcus pneumoniae.*

Ocimium graticismum showed antimicrobial activities at 1 g/ml concentration on *Streptococcus pneumoniae* pathogenic isolate SP1, SP2, SP3 with a greater zone of inhibition on SP3 at 15 mm while it showed a greater zone of inhibition on SP2 at 10 mm and SP1 at 10 mm and this result is similar to 13.5 zone of inhibition observed [28].

Mimosa pudica showed a greater antibacterial activity at 1 g/ml conc with 13 mm zone of inhibition on SP2 followed by SP3 with 10 mm inhibition zone diameter while there was no zone of inhibition at 0.1 g/ml, 0.4 g/ml, 0.6 g/ml and 1 g/ml on SP1. At 0.1 g/ml there were no zone of inhibition on the three clinical isolates

Sida acuta showed a slight effect on the clinical isolate at 0.6 g/ml and 1 g/ml conc on SP1 and SP3 having inhibition zone of 8 mm and 10 mm on 0.6 g/ml and 10 mm and 12 mm on 1 g/ml respectively. Which is similar to 10 mm zone of inhibition observed by Akerele *et al*., (2011) at 0.1 g/ml and 0.4 g/ml concentration; there were no zone of inhibition on the clinical isolates [29].

5. Conclusion

Resistance to commonly used antibiotics and multidrug resistance of S. pneumoniae in the investigated area are remarkably high. Plants have not been completely investigated, nevertheless, data from previous literatures, as well as our results, revealed the great potential of plants for therapeutic treatment. Therefore, more studies need to be conducted to search new compounds. Moreover, once they are extracted and before being used in new therapeutic treatments, they should have their toxicity tested in vivo. However, The plants extract of *Osimum graticismum* showed good antibacterial effects on the organism in high concentration more than other plant extracts while *Sida acuta* and *Newbouldia laevia* plant extract showed weak antibacterial properties to the organism this proves that *Ocimum graticismum* and *Mimosapudica* leaves has good and strong antibacterial properties against streptococcus pneumonia than *Sida acuta* and *Newbouldia laevia* and can be used as an antibacterial agent at adequate or right concentrations.

Declarations

Ethical Approval and Informed Consent

Ethical clearance with reference number (**FETHA/REC/VOL23/ 2023/703)** was obtained from Federal Teaching Hospital Abakiliki. All participants were duly informed of the objectives of the study and the protocol for sample collection. All

participants signed an informed consent form were signed. Participation was voluntary.

Authors Contribution

OEN and IDC conceptualized the study, OOJ designed the study, NOL and COA participated in the fieldwork, K-MOO participated in data collection. OEN prepared the initial draft of the manuscript. All authors contributed to the development of the final manuscripts and approved its submission.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Zafar, M.A., Wang, Y., Hamaguchi, S. and Weiser, J.N. (2017) Host-to-Host Transmission of *Streptococcus pneumoniae* Is Driven by Its Inflammatory Toxin, Pneumolysin. *Cell Host & Microbe*, **21**, 73-83. https://doi.org/10.1016/j.chom.2016.12.005

[2] Obah, I.E., Akerele, J.O. and Obasuyi, O. (2007) Antimicrobial Activity of the Ethanol Extract of the Aerial Parts of Sida Acuta Burm. F. (malvaceae). *Tropical Journal of Pharmaceutical Research*, **6**, 809-813. https://doi.org/10.4314/tjpr.v6i4.14662

[3] Chao, Y., Marks, L.R., Pettigrew, M.M. and Hakansson, A.P. (2015) *Streptococcus pneumoniae* Biofilm Formation and Dispersion during Colonization and Disease. *Frontiers in Cellular* and *Infection Microbiology*, **4**, Article 194. https://doi.org/10.3389/fcimb.2014.00194

[4] Feldman, C. and Anderson, R. (2016) Epidemiology, Virulence Factors and Management of the Pneumococcus. *F*1000 *Research*, **5**, Article 2320. https://doi.org/10.12688/f1000research.9283.1

[5] Fliegauf, M., Sonnen, A.F.-P., Kremer, B. and Henneke, P. (2013) Mucociliary Clearance Defects in a Murine in Vitro Model of Pneumococcal Airway Infection. *PLOS ONE*, **8**, e59925. https://doi.org/10.1371/journal.pone.0059925

[6] Geno, K.A., Gilbert, G.L., Song, J.Y., Skovsted, I.C., Klugman, K.P., Jones, C., *et al.* (2015) Pneumococcal Capsules and Their Types: Past, Present, and Future. *Clinical Microbiology Reviews*, **28**, 871-899. https://doi.org/10.1128/cmr.00024-15

[7] Hackel, M., Lascols, C., Bouchillon, S., Hilton, B., Morgenstern, D. and Purdy, J. (2013) Serotype Prevalence and Antibiotic Resistance in *Streptococcus pneumoniae* Clinical Isolates among Global Populations. *Vaccine*, **31**, 4881-4887. https://doi.org/10.1016/j.vaccine.2013.07.054

[8] Infante, A.J., McCullers, J.A. and Orihuela, C.J. (2015) Mechanisms of Predisposition to Pneumonia. In: *Streptococcus pneumoniae*, Elsevier, 363-382. https://doi.org/10.1016/b978-0-12-410530-0.00019-3

[9] Ejele, A., Ogukwe, C., Duru, I., *et al.* (2012) Phytochemistry and Antimicrobial Potential of Basic Metabolites of *Piper umbellatum*, *Piper guineense*, *Ocimum gratissimium* and *Newbouldia laevis* Extracts. *Journal of Emerging Trends in Engineering* and *Applied Science*, **3**, 309-314

[10] Keller, L.E., Robinson, D.A. and McDaniel, L.S. (2016) Nonencapsulated *Streptococcus pneumoniae*: Emergence and Pathogenesis. *mBio*, **7**, e01792-15. https://doi.org/10.1128/mbio.01792-15

[11] Yahiaoui, R.Y., den Heijer, C.D., van Bijnen, E.M., Paget, W.J., Pringle, M., Goossens, H., *et al.* (2016) Prevalence and Antibiotic Resistance of Commensal *Streptococcus pneumoniae* in Nine European Countries. *Future Microbiology*, **11**, 737-744. https://doi.org/10.2217/fmb-2015-0011

[12] Kim, L., McGee, L., Tomczyk, S. and Beall, B. (2016) Biological and Epidemiological Features of Antibiotic-Resistant *Streptococcus Pneumoniae* in Pre- and Post-Conjugate Vaccine Eras: A United States Perspective. *Clinical Microbiology Reviews*, **29**, 525-552. https://doi.org/10.1128/cmr.00058-15

[13] Lalhminghlui, K. and Jagetia, G.C. (2018) Evaluation of the Free-Radical Scavenging and Antioxidant Activities of Chilauni, *Schima Wallichii* Korth *in Vitro*. *Future Science OA*, **4**, FSO272. https://doi.org/10.4155/fsoa-2017-0086

[14] McMillan, E.A., Gupta, S.K., Williams, L.E., Jové, T., Hiott, L.M., Woodley, T.A., *et al.* (2019) Antimicrobial Resistance Genes, Cassettes, and Plasmids Present in Salmonella Enterica Associated with United States Food Animals. *Frontiers in Microbiology*, **10**, Article 832. https://doi.org/10.3389/fmicb.2019.00832

[15] Tan, S. and Tatsumura, Y. (2015) Alexander Fleming (1881-1955): Discoverer of Penicillin. *Singapore Medical Journal*, **56**, 366-367. https://doi.org/10.11622/smedj.2015105

[16] Tene Tcheghebe, O., Ngouafong Tatong, F., Seukep, A.J., Kamga, J. and Nenwa, J. (2016) Ethnobotanic Survey of Medicinal Plants used for Malaria Therapy in Western Cameroon. *Journal of Medicinal Plants Studies*, **4**, 248-258.

[17] González Mera, I.F., González Falconí, D.E. and Morera Córdova, V. (2019) Secondary Metabolites in Plants: Main Classes, Phytochemical Analysis and Pharmacological Activities. *Bionatura*, **4**, 1000-1009. https://doi.org/10.21931/rb/2019.04.04.11

[18] Muhammad, G., Hussain, M.A., Jantan, I. and Bukhari, S.N.A. (2015) *Mimosa pudica* L., a High-Value Medicinal Plant as a Source of Bioactives for Pharmaceuticals. *Comprehensive Reviews in Food Science* and *Food Safety*, **15**, 303-315. https://doi.org/10.1111/1541-4337.12184

[19] Rabes, A., Suttorp, N. and Opitz, B. (2016) Inflammasomes in Pneumococcal Infection: Innate Immune Sensing and Bacterial Evasion Strategies. In: *Current Topics in Microbiology* and *Immunology*, Springer, 215-227. https://doi.org/10.1007/978-3-319-41171-2_11

[20] Gisch, N., Peters, K., Zähringer, U. and Vollmer, W. (2015) The Pneumococcal Cell Wall. In: *Streptococcus pneumoniae*, Elsevier, 145-167. https://doi.org/10.1016/b978-0-12-410530-0.00008-9

[21] Rai, P., He, F., Kwang, J., Engelward, B.P. and Chow, V.T.K. (2016) Pneumococcal Pneumolysin Induces DNA Damage and Cell Cycle Arrest. *Scientific Reports*, **6**, Article No. 22972. https://doi.org/10.1038/srep22972

[22] Tettelin, H., Chancey, S., Mitchell, T., Denapaite, D., Schähle, Y., Rieger, M., *et al.* (2015) Genomics, Genetic Variation, and Regions of Differences. In: *Streptococcus pneumoniae*, Elsevier, 81-107. https://doi.org/10.1016/b978-0-12-410530-0.00005-3

[23] Trivedi, H.B. and Vediya, S.D. (2013) Removal of Fluoride from Drinking Water with

the Help of *Sidaacuta burm* F. *International Journal of Pharmacy* and *Life Science*, **4**, 2908-2909.

[24] Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. (2018) Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*, **5**, Article 93. https://doi.org/10.3390/medicines5030093

[25] Varnika, S., Ashish, S. and Imran, A. (2012) A Review on Ethnomedical and Traditional Uses of *Mimosa pudica* (Chui-Mui). *International Research Journal of Pharmacy*, **3**, 41-44.

[26] Wilson, R., Cohen, J.M., Jose, R.J., de Vogel, C., Baxendale, H. and Brown, J.S. (2015) Protection against *Streptococcus pneumoniae* Lung Infection after Nasopharyngeal Colonization Requires Both Humoral and Cellular Immune Responses. *Mucosal Immunology*, **8**, 627-639. https://doi.org/10.1038/mi.2014.95

[27] Gonçalves, M.T., Mitchell, T.J. and Lord, J.M. (2015) Immune Ageing and Susceptibility to *Streptococcus pneumoniae*. *Biogerontology*, **17**, 449-465. https://doi.org/10.1007/s10522-015-9614-8

[28] Wintola, O. and Afolayan, A. (2015) The Antibacterial, Phytochemicals and Antioxidants Evaluation of the Root Extracts of Hydnora Africanathunb. Used as Antidysenteric in Eastern Cape Province, South Africa. *BMC Complementary* and *Alternative Medicine*, **15**, Article No. 307. https://doi.org/10.1186/s12906-015-0835-9

[29] Akerele, J., Ayinde, B. and Ngiagah, J. (2011) Phytochemical and Antibacterial Evaluations of the Stem Bark of *Newbouldia laevis* against Isolates from Infected Wounds and Eyes. *Tropical Journal of Pharmaceutical Research*, **10**, 211-218. https://doi.org/10.4314/tjpr.v10i2.66566