Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

Phytochemical and Antimicrobial Analysis of Piper Celtidifirme Opiz and Potential Use as Remedy to Covid-19

Unaro Yau[1], Aezeden Mohamed[2*]
[1]Department of Civil Engineering, Papua New Guinea University of Technology, Lae, Papua New Guinea University, Papua New Guinea.
[2*]Department of Mechanical Engineering, Papua New Guinea University of Technology, Lae, Papua New Guinea University, Papua New Guinea. E-mail: aezeden.mohamed@pnguot.ac.pg

ABSTRACT
This study aims to validate a claim for the traditional use of Piper celtidiformeopiz to cure pneumonia, asthma, and tuberculosis (TB), to establish preliminary data on the phytochemical and antimicrobial investigations, and to delineate how Piper celtidiformeopiz can be considered as a possible cure to COVID-19. Apart from other organic solvents (methanol and chloroform), petroleum ether, due to its greater non-polarity, yields a more specific extract as it only extracts the most polar molecules. Antimicrobial extracts of petroleum ether indicated 81% antimicrobial activity, more significant than the standards (Chloramphenicol and Streptomycin) used. The results have expressed a firm approval on the significance of Piper celtidiformeopiz as a potential treatment to communicable diseases, most notably Klebsiella pneumonia, Streptococcus pneumonia Staphylococcus aureus, the three malicious pathogens that affect the lungs, as observed in the study; which are relative to COVID-19. Evaluation of the results with more emphasis on the bioassay and characterization of phytochemicals signifies their necessity for pharmaceutical research and development.

KEYWORDS
Antimicrobial Activity, Chloroform, Methanol, Petroleum Ether, Piper CeltidiformeOpiz.

Introduction

Coronavirus disease or COVID-19, as subsequently termed by the World Health Organization. Caused by betacoronavirus called severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) [1,2,3]. Emerged in 2019 in Wuhan City, Hubei province in China, the COVID-19 pandemic, an acute infection of the respiratory tract [7], is causing havoc worldwide, infecting more than 42.5 M people with more than 1.14M deaths globally [8]. The disease, though considered to be relative of SARS and the Middle East respiratory syndrome (MERS) [1,3], spreads faster than its two ancestors [3, 5], but its estimated fatality rate is much lower [1, 5]. SARS-CoV-2 affects the lower respiratory tract and manifests as pneumonia in humans [3, 4, 6], with a cluster of illness onsets [6], such as acute respiratory distress syndrome (ARDS) and multi-organ dysfunction [5, 6], resulting in common symptoms such as excessive inflammation of the lower airways [9].

The novel coronavirus was rampant, as is evident everywhere globally, and scientists endeavored to discover potential drugs for its cure. To date, the investigation for a potential cure is ongoing, with no specific drugs for efficacious treatment [10, 11]. Clinical trials conducted in China regard chloroquine phosphate, a new drug for malaria treatment, as useful and acceptably safe against COVID-19 [10], which is dubious [10, 11].

Can herbal plants be an alternate treatment to this quandary? Throughout history, men have always turned to nature to seek answers to their travailing questions. Traditional medicinal plants display a broad spectrum of phytochemicals to treat chronic and infectious diseases [12, 13, 14]. Relative to this survival mode that extends throughout the globe, PNG has embarked primarily on its natural environment apart from the significant spiritual concepts to attain cure [15]. PNGs lifestyle intertwined with the plant and animal life of the country. Claiming that Piper CeltidiformeOpiz, a native plant species to Papuasia, has been used in the Baluan Island, Manus Province, to treat tuberculosis (TB), asthma, and pneumonia [15]. These three malignant diseases are common lung infections [16, 17, 18], relative to COVID-19, as discussed above.

The claim should be validated through thorough investigation and the establishment of preliminary data on the phytochemical and antimicrobial assessment of Piper celtidiformeopiz, to verify its traditional use to treat pneumonia, asthma, and TB how it can also consider as a possible cure to COVID-19.

The study incorporates Sustainable Technology because of the technology used for sample extractions and subsequent analysis. Of course, the local availability of the plant species makes the study sustainable.

Materials and Methods

The following are materials and their procedure; sampling, plant extract preparation, phytochemical screening, and antimicrobial screening will discuss in detail.

Sampling

A sufficient quantity of fresh leaves and stems identify and collected from Piper celtidiformeopiz in Gabmutzung (Bulolo). Placed in a labeled open paper bag and transported to the laboratory. Rinsed thoroughly with sterile distilled water (excess water blotted out with a paper towel). Air-dried for three days; then grounded in a stainless-steel mill and collected in a labeled plastic bag; and then finally referred to extraction and further phytochemical and antimicrobial screening.

Plant Extract Preparation

Thirty grams of the grounded leaf and stem samples of Piper celtidiformeopiz were extracted separately with 300 mL of methanol, chloroform, and petroleum ether respectively by soaking overnight. Separate filtrates were then collected in sampling bottles and stored for subsequent phytochemical and antimicrobial screening.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

Phytochemical Screening

- **Reducing Sugars Test**

Reducing sugars test or Fehling's test consist of a few drops of methanol were added to boiling Fehling's solution (A and B). The formation of a red precipitate indicates a positive test for reducing sugar.

- **Terpenoids Test**

Terpenoids test or Salkowski test consist of 2 mL of chloroform was added to 0.5 grams of the extracts, followed by carefully adding 3 mL of concentrated sulphuric was acid. A reddish-brown coloration of the interface layer indicates the presence of terpenoids.

- **Flavonoids Test**

5 mL of dilute ammonia (6M) added to a portion of the extract's aqueous filtrate to produce the solution's yellow color. The yellow color disappeared when left to stand, denotes flavonoids after concentrated sulphuric acid (1 ml) was added.

- **Saponins Test**

The extract's aqueous filtrate was diluted with distilled water to 20 mL and shaken in a test tube for 15 minutes. The presence of saponins denoted by the formation of a 1 cm layer of foam.

- **Saponins Test**

The extract's aqueous filtrate was diluted with distilled water to 20 mL and shaken in a test tube for 15 minutes. The presence of saponins denoted by the formation of a 1 cm layer of foam.

- **Tannins Test**

A few drops of 1% lead acetate were added to 5.0 ml of the extract's aqueous filtrate. The formation of yellow precipitate denoted the presence of tannins.

- **Alkaloids Test**

0.5 mL of aqueous filtrate of the extract was diluted to 10 mL with ethanol, boiled, and filtered. 2 mL of dilute ammonia (6M) was added to 5 ml of the filtrate. To this, 5 ml of chloroform was added and gently agitated to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid, divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of creamy suspension (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent) denotes the presence of alkaloids.

- **Steroids Test**

1 mL of aqueous filtrate of the extract was dissolved in 10 ml of chloroform, and 10 mL of concentrated sulphuric acid was added to the inside walls of the test tube. The presence of steroids was noted by the formation of the red upper layer and sulphuric acid layer showing yellow with green fluorescence.

Antimicrobial Screening

- **Materials and Reagents**

Materials used in microbiology screening include the everyday laboratory materials required for the agar overlay technique. The microorganisms tested were obtained from respective stock cultures. These pathogenic microorganisms were prepared by transferring a loop full of microorganisms from each stock culture into labeled nutrient broths and incubated at respective temperatures for each microorganism 24 hours [13].

- **Procedure**

The experimental procedure steps as following:

a) 6 mm paper discs were prepared by punching the millipore filter papers and placing them in the Petri-disc according to the number of microorganisms tested. The Petri disc containing each plant extracts was clearly labeled.
b) 11.5 grams of universal nutrient agar were weighed and transferred into a 500 mL Pyrex bottle and dissolved by adding 500 mL of water. The solution was then sterilized in an autoclave for about 15 minutes at 121^{o}C.
c) After autoclaving, the agar bottle was then placed in a water bath set at 50^{o}C. Then the agar was cooled and poured into the labeled sterile Petri-discs and allowed to stand to solidify.
d) 30 μL of the combined plant extract was then transferred onto paper discs while the agar is solidifying. After the nutrient agar solidified, each plan extract's stained paper discs will then be transferred to individual discs and allowed to dry.
e) The microorganisms from the nutrient broth was then added and spread evenly onto the solidified agar on the petri-dish using a glass hockey rod.
f) Impregnated paper discs were then placed onto the seeded media and incubated at respective temperatures 24 hours.
g) The antimicrobial activities were then determined by measuring the inhibition zone's diameter (mm) around the paper discs.

Results and Discussion

Studies on plant-derived molecules continue to amaze researchers with their promising therapeutic effects. Among the plants investigated to date, one showing enormous potential is the Piper species (The family name for Piper celtidiformeopiz). Pipers appreciate the world as some useful medicinal plants, whose therapeutic attributes are perplexing, considering their phenomenal characters as remedies for different diseases at different localities. Traditional

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

societies in tropical countries use many genus Piper species worldwide as stimulants, antiseptics, anti-inflammatories, analgesics, antioxidants, and medicines [12]. With such a richly proliferated history, Piper celtidiformeopiz has immense potential for pharmaceutical research and development.

Methanol, chloroform, and petroleum ether were the organic solvents used for the total extraction due to their high solubility.
The methanol extract showed saponins, steroids, tannins, alkaloids, flavonoids, and reducing sugars, with alkaloids and flavonoids being dominant and consistent for both stem and leaf samples. Chloroform extract showed similar results as methanol extract but with strong indications for steroids, tannins, and flavonoids. Whereas with petroleum ether, steroids, alkaloids, flavonoids, and reducing sugars are highly dominant and consistent in sample extract (Table 1).

Table 1. Phytochemical test result for Piper celtidiformeopiz plant extracts

Phytochemical Test Results								
Solvent	Sample	Saponins	Triterpenoids	Steroids	Tannins	Alkaloids	Flavonoids	Reducing Sugars
Methanol	S3 Leaf	++	-	-	+++	++	+++	+++
	S3 Stem	+	-	++	-	+++	+++	-
Chloroform	S3 Leaf	++	-	++	+++	+++	++	-
	S3 Stem	+	-	+++	++	-	+++	-
Petroleum ether	S3 Leaf	++	+++	++	-	+	+++	+++
	S3 Stem	-	-	+++	+	+++	+++	+++

The antimicrobial activity of Piper celtidiformeopiz showed that; Methanol extracts were active against all pathogens, except for Escherichia coli (Figure 1). Chloroform extracts only performed against Proteus Vulgaris and Micrococcus Luteus but resisted by the rest of the pathogens (Figure 1).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

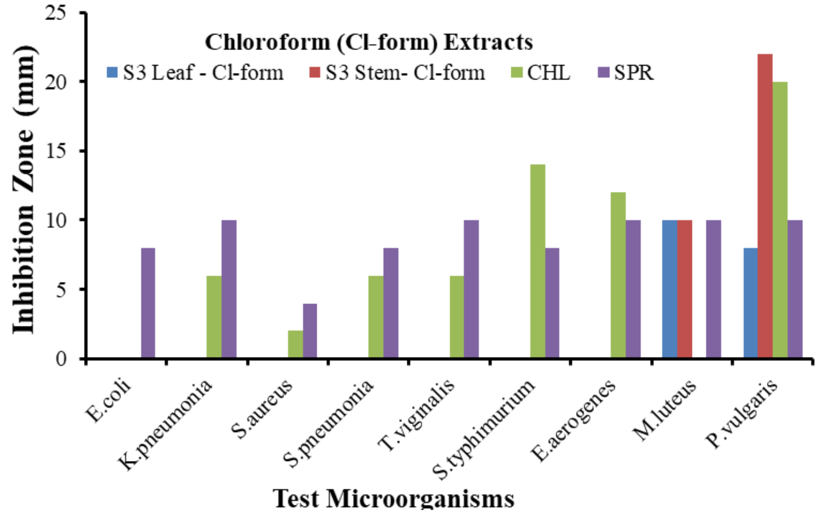

Figure 1. Antimicrobial activity with Chloroform extracts

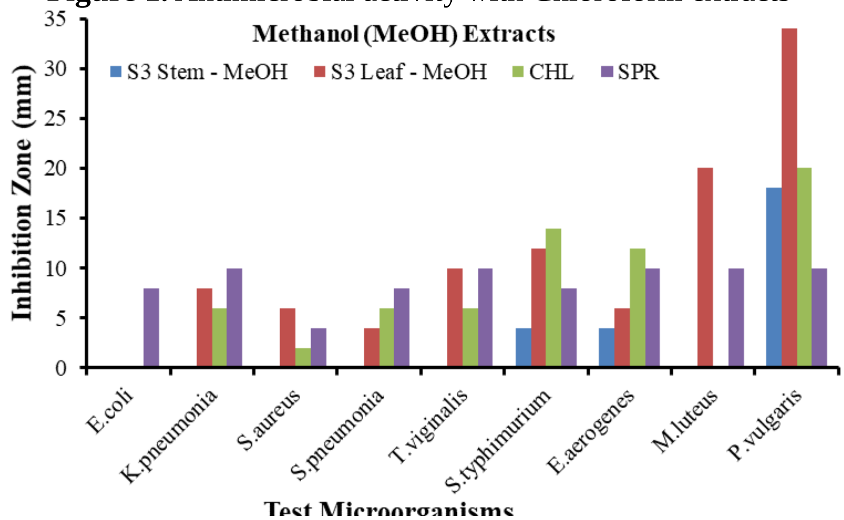

Figure 2. Antimicrobial activity with Methanol extracts

For the Petroleum ether extracts showed remarkable activity against all nine pathogens studied (Figure 3 and 4).

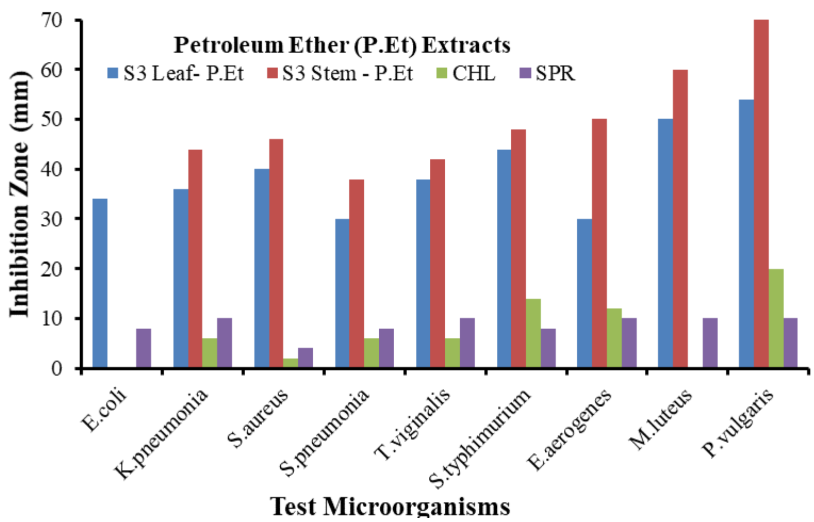

Figure 3. Antimicrobial activity with Petroleum Ether extracts

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

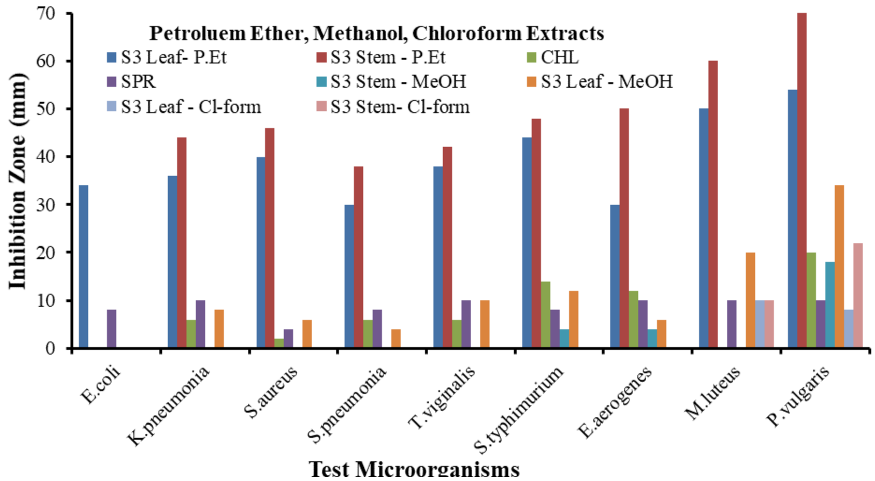

Figure 4. Antimicrobial activity for Chloroform, Methanol, and Petroleum Ether extracts

Results and Discussion

In this study, plant extract's phytochemical and antimicrobial activity showed remarkable results, displaying its therapeutic essence. The flavonoid is most dominant in all parts and analyzed from the phytochemical results obtained, followed by alkaloid, steroids, tannins. They are reducing sugars, saponins, and triterpenoids.
The bioactive chemical compound present is relative to the poisoning of the microorganisms, thus inhibiting them from their activities in two ways as follow:

a) Directly; disrupting one or more functions in their metabolic processes.
b) Indirectly; changing the environmental conditions or inhibiting their food sources.

Phytochemical analysis of any plant parts may be the most vital aspect of research's pharmacological significance. Identifying the compound responsible for the toxicity of the microorganisms facilitating the common major diseases relies firstly on determining the major classes of compounds known to have different chemical properties.
The microbial screening also produces results that are eloquent in deriving subsequent substitute for the plant extracts. The activity of the plant extract's toxicity to the microorganisms tested is proportional to the inhibition zone (IZ). The higher inhibition zone is consequently relative to higher activity due to the higher concentration of the toxic bioactive compound. The standards chloramphenicol (CHL) and streptomycin (SRP) are used as reference material for comparison purposes.

In this broad survey, nine microorganisms tested were typically the gram-negative, gram-positive protozoa.Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Micrococcus lutea, Proteus Vulgaris, Pseudomonas florescens, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pneumonia, and Trichomonas viginalis.

The relevance here amplifies how active the toxicity is and how extensive the toxicity can go. The results have expressed a firm approval on the significance of Piper celtidiformeopiz as a potential treatment to communicable diseases, most notably Klebsiella pneumonia, Streptococcus

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

pneumonia Staphylococcus aureus. These three malicious pathogens affect the lungs, which is relative to COVID-19.

As illustrated in Table 1, methanol extracts showed consistency in saponins, alkaloids, and flavonoids. The antimicrobial activity of the extracts was exceptional against Proteus Vulgaris (IZ=34mm). Micrococcus Luteus (IZ=20mm) and performed relative to the standards used for the other pathogens, except for Escherichia coli, which demonstrate nil antimicrobial activity (i.e., resistant to the toxicity of the bioactive compounds present in the extract) (Figure 1).

Chloroform extracts with high saponins, steroids, flavonoids, and reducing sugar (Table 1) seemed to perform well against Proteus Vulgaris (IZ=22mm) and Micrococcus Luteus (IZ=10mm), as compared to the standards. However, the rest of the microorganisms were resistant to extract's antimicrobial activity (Figure 2).

On the other hand, Petroleum Ether extracts showed a high degree of saponins, triterpenoids, steroids, flavonoids, and reducing sugars from the leaf extracts. In contrast, the stem extract showed similar results, except for saponins. Antimicrobial analysis of petroleum extracts shows extraordinary antimicrobial results, which were quite perplexing. Both the stem and leaf extracts perform remarkably well, surpassing the standards used for all the pathogens, except for Escherichia coli, which was resistant to extracts' antimicrobial activity from the stem.

Conclusion and Recommendation

Despite the variation in yield, there was no concrete significance of variation in the chemical constituents present and the degree of their presence. The phenomenal antimicrobial results were more effective when petroleum ether was used as the extraction solvent. The stem probably is the potential part but can be synergized with the leaf to magnify the extract's therapeutic effects.

The controversial scenario of the antimicrobial results obtained may be due to the different solubility power of these different solvents utilized in this study. Due to its greater non-polarity, Petroleum ether yielded a more specific extract than chloroform and methanol because it will only extract the most polar bioactive compounds, hence higher antimicrobial activity.

The bioactive chemical is responsible for flavonoids' antimicrobial activity (being the most predominant chemical group). With some synergistic effects from another chemical group (s), especially the alkaloid group present, and steroids, since these three chemical groups are dominant in petroleum extract of; hence producing extraordinary results.
Due to the enormous degree of activity indicated by the petroleum extract, further research on bioassay isolation and identifying these active constituents most recommended. Evaluation of the results with more emphasis on the bioassay and characterization of phytochemicals signifies their necessity for pharmaceutical research and development.

References

[1] He, Y., Wang, J., Li, F., & Shi, Y. (2020). Main clinical features of COVID-19 and potential prognostic and therapeutic value of the microbiota in SARS-CoV-2 infections. *Frontiers in Microbiology*, *11*, 1302. https://doi.org/10.3389/fmicb.2020.01302

[2] Rothan, H.A., &Byrareddy, S.N. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *InJournal of Autoimmunity*, *109*, 102433. https://doi.org/10.1016/j.jaut.2020.102433

[3] Sohrabi, C., Alsafi, Z., O'Neill, N., Khan, M., Kerwan, A., Al-Jabir, A., Iosifidis, C., & Agha, R. (2020). World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). *InInternational Journal of Surgery*, *76*, 71-76. https://doi.org/10.1016/j.ijsu.2020.02.034

[4] Sheng, W.H. (2020). Coronavirus disease 2019 (covid-19). *Journal of Internal Medicine of Taiwan*, *31*(2), 61-66. https://doi.org/10.6314/JIMT.202004

[5] Singhal, T. (2020). A Review of Coronavirus Disease-2019 (COVID-19). *InIndian Journal of Pediatrics*. *87*, 281–286.https://doi.org/10.1007/s12098-020-03263-6

[6] Zhao, D., Yao, F., Wang, L., Zheng, L., Gao, Y., Ye, J., Guo, F., Zhao, H., & Gao, R. (2020). A Comparative Study on the Clinical Features of Coronavirus 2019 (COVID-19) Pneumonia with Other Pneumonias. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *71*(15), 756–761. https://doi.org/10.1093/cid/ciaa247

[7] Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., &Wendtner, C. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature*, *581*, 465–469. https://doi.org/10.1038/s41586-020-2196-x

[8] Dong, E., Du, H., & Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. *The Lancet infectious diseases*, *20*(5), 533-534. https://doi.org/10.1016/S1473-3099(20)30120-1

[9] Parrot, T., Gorin, J.B., Ponzetta, A., Maleki, K.T., Kammann, T., Emgård, J., & Sandberg, J.K. (2020). MAIT cell activation and dynamics associated with COVID-19 disease severity. *Science immunology*, *5*(51). https://doi.org/10.1126/sciimmunol.abe1670

[10] Gao, J., Tian, Z., & Yang, X. (2020). Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *In Bioscience Trends*, *14*(1), 72-73. https://doi.org/10.5582/BST.2020.01047

[11] Yavuz, S., &Ünal, S. (2020). Antiviral treatment of COVID-19. *Turkish journal of medical sciences*, *50* (SI-1), 611-619. https://doi.org/10.3906/sag-2004-145

[12] Duraipandiyan, V., Ayyanar, M., &Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC complementary and alternative medicine*, *6*(1), *35–41*.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 388 - 397
Received 16 February 2021; Accepted 08 March 2021.

[13] Cos, P., Vlietinck, A.J., Berghe, D.V., &Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *Journal of ethnopharmacology*, *106*(3), 290-302.

[14] Campion, K. (1988). *The Family Medicinal Herbal*. Australia, Collins Publishers, 56-57.

[15] Woodley, H. (Ed, 1991). *Medicinal Plants of Papua New Guinea*: *Morobe Province.* (Verlag Josef Margraf, Germany).

[16] Periselneris, J.N., Brown, J.S., & José, R.J. (2020). Pneumonia. *In Medicine (United Kingdom),48*(6), 351-355. https://doi.org/10.1016/j.mpmed.2020.03.002

[17] Fogel, N. (2015). Tuberculosis: A disease without boundaries. *InTuberculosis*, *95*(5), 527-531. https://doi.org/10.1016/j.tube.2015.05.017

[18] Holgate, S.T., Wenzel, S., Postma, D.S., Weiss, S.T., Renz, H., & Sly, P.D. (2015). Asthma. *Nature Reviews Disease Primers*, *1*, 15025. https://doi.org/10.1038/nrdp.2015.25

[19] Phytochemical screening and Extraction. (2011).InternationalePharmaceuticaSciencia, 105, 1. http://www.ipharmsciencia.com © 2011 IPS.