Nigerian Journal of Pharmaceutical and Applied Science Research
Vol. 13 (1): 19-25; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net; https://doi.org/10.60787/nijophasr-v13-i1-531

Phytochemical, Antioxidant and Antimicrobial Evaluation of Nigerian *Kalanchoe pinnata* (Lam.) Roots.

***[1]Christiana A. Kendeson, [2]Mary L. Kagoro and [2]Esther A. Adelakun**

[1]Department of Chemical Sciences, Federal University of Kashere, Gombe, Nigeria.
[2]Department of Chemistry, University of Jos, Jos, Nigeria.

ABSTRACT

***Background*:** *Kalanchoe pinnata* (Lam.) is a plant belonging to the family Crassulaceae or orpine family and reported to possess medicinal properties. This study was undertaken to investigate the phytochemical profile, as well as the antioxidant and antimicrobial properties of the Nigerian *Kalanchoe pinnata* (Lam.) root extract.

***Methods*:** The roots of the plant (300 g) were extracted with 70 % ethanol. The quantitative and qualitative phytochemical screening was done following standard methods. The antioxidant activity was determined by evaluating the scavenging activity using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical assay and the antimicrobial activity was determined using the agar well diffusion method.

Results: The percentage yield of the extract was found to be 3.79 %. From the phytochemical screening alkaloids, flavonoids, glycosides, phenolics, saponins, steroids/terpenes and tannins were detected. The quantitative analysis gave the phenolic content as 3.800 ± 0.023 mg gallic acid equivalent; flavonoid content as 0.277 ± 0.004 mg quercetin equivalent and tannin content as 0.017 ± 0.057 mg tannic acid equivalent. The antioxidant assay result indicated that the plant exhibited antioxidant activity with IC_{50} value of 40.56 and 35.47 µg/mL for ascorbic acid and root extract respectively. The antimicrobial activity results showed that the crude extract inhibited the growth of the test microorganisms *Bacillus cereus*, *Echerichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Candida albicans*; while the extract was inactive against *Salmonella typhi*.

Conclusion: These findings indicate that the plant possesses antioxidant and antimicrobial properties which could be due to the presence of the phyto-compounds present in the plant.

Keywords: Antioxidant, Antimicrobial, *Kalanchoe pinnata*, Phytochemical, Root.

1. INTRODUCTION

Most plants are said to have therapeutic potential, which is attributed to phytochemicals, which are used as agents in pharmacological research and medication development [1]. This has led to more quests for their advanced biological activities such as antioxidants, antimicrobial, anti-inflammatory, anti-ulcer, anticancer activities, etc. [2]. Most of these phytochemicals work in synergy and serve as health protective shields, each having a specific and distinct role in the defense of the plant. These include alkaloids, polyphenols, terpenoids, saponins and polypeptides which are known to reduce oxidative stress through free radical scavenging capacity as well as inflammation, and antimicrobial activities [3, 4, 5]. However, it is worthy of note that the activity identified by an *in vitro* test does not necessarily confirm that a plant extract is an effective medicine, nor a suitable candidate for drug development but it does provide basic understanding of a plant's efficacy and, in some cases toxicity [6]. *Artemisia annua*, *Cincona officinalis* and

***Corresponding author: Email: kendesonac@yahoo.com; Phone: +2348065687565**

Camptotheca acuminate are among plants that have been proven to contain phytochemical compounds from which artemisinin, quinine and camptothecin were isolated respectively, and subsequently drugs (antimalarial and anticancer) developed from them [7, 8, 9]. *Moringa oleifera*, *Polyalthia longifolia* and *Antidesma venosum* are some of the plants containing phytochemical compounds whose herbal remedies have been reported to pose no danger to biodiversity but have not been standardized for use as drugs [6, 10]. *Kalanchoe pinnata* is known to possess active phytochemical constituents such as alkaloids, polyphenols, terpenoids, steroids, saponins etc [11, 12, 13] which could be responsible for its various activities reported by various literatures such as: antimicrobial [14], antioxidant [11], anti-inflammatory [15, 16, 17], analgesic activity [18], etc. This paper reports the phytoconstituents and the biological properties of *K. pinnata* root obtained from Gboko L.G.A of Benue State, Nigeria. This is a preliminary investigation to provide scientific evidence for the use of the plant in the treatment of infections and ulcers by the locals.

2. MATERIALS AND METHODS

2.1 Materials

All reagents and chemicals were of analytical grade and commercially purchased. UV/Vis spectrophotometer (Cecil CE 7400, 7000 series) was used to obtain the absorbance of analytes. Nutrient agar, potatoes dextrose agar and Mueller Hinton broth, ascorbic acid, gallic acid, quercetin**,** tannic acid, 1,1-diphenyl-2-picryl hydrazyl (DPPH) were obtained from Sigma-Aldrich, Germany.

2.2 Methods

2.2.1 Collection of plant sample

K. pinnata (Lam) plants were collected from Yandev, Gboko L.G.A, Benue State, Nigeria, with a geographic location of Longitude 9º 02' 34.84" E and Latitude 7º 21' 40.71" N. Identification of the plant was done at the Federal college of Forestry, Jos, Nigeria and a specimen deposited at the herbarium with voucher number FHJ 263. The fresh roots were cut, cleaned, air dried and the dried plant materials were stored in sterile containers for further use.

2.2.2 Extraction

The crushed roots (300 g) were macerated using 70 % ethanol with frequent agitation to improve the extraction efficiency. The extract was filtered, concentrated using rotary evaporator at 40 ºC and dried in an evaporating dish on a water bath at 40 ºC to obtain a brownish solvent-free crude extract. The percentage yield of the extract was calculated and noted.

2.2.3 Phytochemical analysis

The extract was screened for the presence of various phytochemical tests following standard methods described by Sofowora [19], Harborne [20], Trease and Evans [21] for the presence of compounds such as alkaloids, flavonoids, steroids/terpenes, glycosides, saponins, tannins and phenols. The quantitative analysis for total flavonoids, phenols and tannins contents were carried out as described by Kaur and Kapoor [22] and John *et al*., [23].

2.2.4 Antioxidant assay

*Determination of DPPH radicals scavenging activity***:** The free-radical scavenging activity was determined using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) method described by Kendeson *et al*., [13]. 0.1 mM solution of DPPH in ethanol was prepared, and 1.5 mL of this solution was added to 1.5 mL of the standard/sample extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/mL). The mixture was shaken vigorously using a vortex mixer (SA7) and allowed to stand at room temperature for half an hour then, absorbance was measured at 517 nm using UV/Vis spectrophotometer (Cecil CE 7400, 7000 series). Ascorbic acid was used as a standard reference compound. The percent DPPH scavenging effect was calculated by using equation:

Percent (%) inhibition = $A_0 – A_1 / A_0 \times 100$.

Where A_0 = the Absorbance of control and A_1 = the Absorbance of standard/sample.

All measurements of free radical scavenging activity were performed in triplicate and standard deviation was calculated. The concentrations of samples resulting in 50 % inhibition on DPPH (IC_{50} value) was calculated by getting a linear regression between the plot of concentrations and the percentage inhibitions, so that the regression equation

given as y = mx + c was obtained, then y was replaced with the value 50, so it became 50 = mx + c, and x = (50-c)/m. The value of x obtained was the IC_{50} value.

2.2.5 Antimicrobial activity

Standard and clinically isolated microorganism strains namely: *Salmonella typhi*, *Bacillus cereus*, *Echerichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albicans*, and *Aspergillus niger*; were obtained from Federal Teaching Hospital, Gombe, Gombe State. These were used to test for the *in vitro* antimicrobial activity of the crude extract as described by Sanchez *et al*., [24], Ochei and Kolhatkar [25]. The antimicrobial activity of the crude extract was determined by using the agar well diffusion method. Standardization of cultures was carried out by suspending some colonies of an overnight culture of the bacteria and two night's culture of the fungus in 5 mL of nutrient broth and comparing the turbidity with that of 0.5 MacFarland turbidity standards after incubating at 37 ºC for 6 hours and 12 hours for the bacteria and fungi respectively. The test organisms (l mL) were poured into sterile Petri dish and 25 mL of nutrient agar/potatoes dextrose agar added, mixed well, allowed cooling and solidifying at room temperature with the lids closed. 6 wells each of 4 mm in diameter were bored on the seeded agar using sterilized stainless steel cork borer. The cork borer was sterilized using the flame after each well was bored. 5 out of the 6 wells were filled with various concentrations of the crude extracts, while the 6^{th} well was filled as the control. The plates were left to stand for 30 minutes to pre-diffuse and then incubated at 37 ºC for 24 hours. The fungi were incubated at 37 ºC for 48 hours and the diameters of the zones of inhibition were measured in millimeter using a transparent metre rule. The minimum inhibitory concentration (MIC) of the crude extract was determined for each of the isolates at varying concentrations (200, 100, 50, 25 and 12.5 mg/mL). To obtain these concentrations, l mL of each extract containing double the strength of the concentrations (200, 100, 50, 25 and 12.5 mg/mL) were placed in test tubes. 1 mL of Mueller Hinton broth was added and 0.2 mL test organism introduced into the test tubes. The test tubes were properly swirled, plug with cotton wool and covered with aluminium foil. All the tubes were incubated at 37 ºC for 24 hours for the bacteria and 37 ºC for 48 hours for the fungi, after which they were examined for growth. The MIC of the extract was the lowest concentration of the extract that inhibited the growth of the specified inoculums of the test organisms. The minimum bactericidal/ fungicidal concentration (MBC/MFC) was determined by pipetting 0.2 mL of the culture from the test tubes that showed no growth during the MIC determination and inoculated on nutrient/potatoes agar plates that contained no antibiotic or extract. These were inoculated at 37 ºC for 24 hours for the bacteria and 37 ºC for 48 hours for the fungi. The concentration that showed no growth was taken as the MBC/MFC.

3. RESULT

The percentage yield of the crude extract of *K. pinnata* roots was found to be 3.79 %. The results of phytochemical analysis; phenolic, flavonoid and tannin contents; DPPH analysis and antimicrobial activity of the crude extract of *K. pinnata* roots are shown in Tables 1 - 4, respectively.

Table 1: Results of Phytochemical Analysis of Crude Extract of the Root *K. pinnata*

Phytochemical Test	Observation
Alkaloids	+
Flavonoids	+
Glycosides	+
Phenolics	+
Saponins	+
Sterols	+
Terpenes	+
Tannins	+

Key: + indicates presence of the constituents.

Table 2: Total Phenolic, Flavonoid and Tannin contents in Crude Extract of *K. pinnata*

Phytochemical	Value
Phenolic content (mg GAE/g)	5.538 ± 0.005
Flavonoid content (mg QE/g)	0.242 ± 0.001
Tannin content (mg TAE/g)	0.019 ± 0.001

Values are mean ± SD of three replicates.

Table 3: DPPH assay of Ascorbic Acid and Crude Extract of *K. pinnata*

Concentration (µg/ml)	% Inhibition	
	Ascorbic acid	Crude extract
5	12.14	10.28
10	17.29	14.14
12	23.43	20.86
20	27.57	28.29
25	31.71	37.71
30	36.14	41.29

IC_{50} for ascorbic acid and the root crude extract is 40.56 and 35.47 µg/mL respectively

Table 4: Antimicrobial Activity of Crude Extract of *K. pinnata* Root

Test Organisms	Zones of Inhibition (mm)						MIC (mg/mL)	MBC/MFC (mg/mL)
	Concentration of Extracts (mg/mL)							
	200	100	50	25	12.5	C		
B. cereus	25	20	14	8	-	22	50	200
E. coli	28	21	13	6	-	23	100	200
S. typhi	-	-	-	-	-	-	-	-
S. aureus	28	24	19	13	-	24	100	200
P. aeruginosa	22	17	10	6	-	23	100	200
K. pneumonia	30	24	17	10	8	26	50	-
C. albicans	10	6	-	-	-	11	-	-
A. niger	32	27	24	-	-	20	100	200

Key**:** - implies no sensitivity
MIC = Minimum Inhibitory Concentration
MBC = Minimum Bactericidal Concentration
MFC = Minimum Fungicidal Concentration
C = control (standard drug)
Ciprofloxacin (10 mg/mL) for bacteria
Ketoconazole (10 mg/mL) for fungi

4. DISCUSSION

The phytochemical screening showed the presence of constituents such as alkaloids, flavonoids**,** glycosides**,** phenolic compounds**,** saponins**,** sterols, terpenes and tannins (Table 1), similar results have been reported for the leaf and stem parts of the plant by Okwu & Josiah, [25]; Majaz *et al*., [27]; Kendeson *et al*., [12, 13]. It was observed that the phenolic content (3.800 ± 0.023 mg GAE/g of extract) was higher than the sum of the flavonoid content (0.277 ± 0.004 mg QE) and tannin content (0.017 ± 0.057 mg TAE) as shown in Table 2. This suggests that the root contains other compounds with the phenolic group, other than flavonoids and tannins which were detected in the phytochemical screening. These phytochemicals might be responsible for its biological activities, especially flavonoids. Phenols, as well as polyphenolic compounds which include flavonoids and tannins play a significant role in providing defensive and fungicidal actions in plants as well as act as antioxidants [2, 28]. Alkaloids have remarkable diversity of medicinal applications and are well-known for their wide range of pharmacological properties, including, but not limited to, anti-microbial, anti-hypertensive, anti-malarial, antidepressant, anti-inflammatory, emetic, diuretic, and anti-cancer effects [26, 29]. Saponins protect against hypercholesterolemia and antibiotic properties; steroids and terpenoids show the analgesic properties [30]. The antioxidant activity of the root crude extract was evaluated using DPPH assay with ascorbic acid as standard and the result showed that the half-maximal inhibitory concentration (IC_{50}) values of the ascorbic acid and root extract were 40.56 and 35.47 µg/mL, respectively (Table 3), implying that the root crude extract exhibited higher activity than the ascorbic acid. DPPH assay provides insight into the potency or otherwise of a sample against free radical species which adversely affect nucleic acids, polysaccharides and lipids in living systems [31]. The antioxidant property exhibited by the extract may be due to the phyto-compounds of the extract which include the flavonoids and phenols with reported antioxidant effect [32]. The antimicrobial activities of the crude extract showed that it was concentration dependant and inhibited the growth of *Bacillus cereus*, *Echerichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Candida albicans* while *Salmonella typhi* showed resistance (Table 4). This has shown that the extract was active against some gram negative bacteria (*P. aeruginosa* and *K. pneumoniae*) as well as gram positive bacteria (*B. cereus*, *E. coli* and *S. aureus*) making it a good candidate for a broad spectrum antimicrobial agent; it could also serve as antifungal agent, since *A. niger* and *C. albicans* are fungal agents The antimicrobial activity exhibited by the root extract may be

attributed to the secondary metabolites – alkaloids, flavonoids, glycosides, phenolics**,** saponins, steroids, terpenoids and tannins, which were detected in the extract and are reported to demonstrate activity against micro-organisms [29, 2]. The findings in this work agree with previous work done on the stem-bark and leaves reported by Kendeson *et al*., (12, 13).

5. CONCLUSION

According to this study, the ethanolic root extract contains a number of phytochemical components that have been identified. It also highlights the anti-oxidant and anti-microbial properties of the extract against specific micro-organisms. This has demonstrated that the Nigerian *K. pinnata* (Lam.) has bioactive constituent(s) that may account for its ethnomedical applications by the natives of Gboko Local Government Area of Benue State. To identify and characterize the constituent(s), more research is being conducted.

Acknowledgement
The authors are grateful to Tertiary Education Trust Fund-Nigeria (TETFund) for the fellowship sponsor for Christiana A. Kendeson

Conflict of Interest
The authors declare no conflicts of interest.

Contribution of the Authors
Significant contributions to the study's conceptualization, planning, and implementation came from Christiana A. Kendeson, Mary L. Kagoro, and Esther A. Adelakun. All laboratory experiments and research were conducted by Christiana A. Kendeson under the guidance of Mary L. Kagoro and Esther A. Adelakun. Christiana A. Kendeson handled the research, data collecting, analysis, and writing of the article. Mary L. Kagoro and Esther A. Adelakun oversaw the entire writing process, from concept to final manuscript clearance. Each author reviewed and gave their approval to the completed work.

6. REFERENCES

[1] Ekeocha, P. A., Ezeh, C. O., Anyam, J. V., Onyekwelu, C. K., Ikekpeazu, J. and Igoli J. O. (2021). Isolation, structural elucidation and therapeutic potentials of root of *Cucurbita pepo*. *Indian J Pharm Sci*, *83*(6): 1288 - 1294. DOI: 10.36468/pharmaceutical-sciences.884.

[2] Egbuna, C., Ifemeje, C. J., Udedi, C. S. & Kumar, S. (Eds.). (2019). Phytochemistry: fundamentals, modern techniques and applications (Vol. 1). Waretown: Apple Academic Press. Pp. 4 - 18.

[3] Hossen, I., Hua, W., Ting, L., Mehmood, A., Jingyi, S., Duoxia, X., Yanping, C., Hongqing, W., Zhipeng, G., Kaiqi, Z., Fang, Y. & Junsong, X. (2019). Phytochemicals and inflammatory bowel disease: A Review, *Critical Reviews in Food Science and Nutrition*, *60*(8), 1321-1345. DOI: 10.1080/10408398.2019.1570913.

[4] Maulana, T. I., Falah, S. & Andrianto, D. (2019). Total phenolic content, total flavonoid content, and antioxidant activity of water and ethanol extract from Surian (*Toona sinensis*) leaves. *IOP Conf. Series: Earth and Environmental Science*, *299*(1): 1 - 10.

[5] Al Sheikh, H. M. A.; Sultan, I.; Kumar, V.; Rather, I. A.; Al-Sheikh, H.; Tasleem ,J. A.; Haq, Q. M. R. (2020). Plant-Based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics*, *9*(8): 480. http://doi.org/10.3390 /antibiotics9080480

[6] Iqbal, A., Farrukh, A. & Mohammad, O. (2006). Modern Phytomedicine: Turning Medicinal Plants into Drugs. WILEY-VCH Verlag GmbH & Co. KGaA,*Weinheim*; 20 - 21 p.

[7] Kinghorn, A. D. & Balandrin, M. F. (1993). *Artemisia annua*. Human medicinal agents from plants, [*ACS Symposium Series*]; American Chemical Society: Washington, DC, 525: 242 - 255.

[8] Ajah, O. P. & Eteng, U. M. (2010). Phytochemical screening and histopathological effects of single acute dose administration of *Artemisia annua* L. on testes and ovaries of Wistar rats. *African Journal of Biochemistry Research*, *4*(7): 179 - 185. http://doi.org/10.5897/AJBR.9000187

[9] Kumar, A., Patil, D., Rajamohanan, P. R. & Ahmad, A. (2013). Isolation, purification and characterization of Vinblastine and Vincristine from endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus. PLoSONE*, *8*(9): 1 - 10. DOI: 10.1371/journal.pone.0071805

[10] Ebiloma, G. U., Igoli, J. O., Katsoulis, E., Donachie, A. M., Eze, A., Gray, A. I. & de Koning, H. P. (2017). Bioassay-guided isolation of active principles from Nigerian medicinal plants identifies new trypanocides with low toxicity and no cross-resistance to diamidines and arsenicals. *Journal of Ethnopharmacology*, *202*: 256 - 264. doi.org/10.1016/j.jep.2017.03.028

[11] Onoja, O. S., Ihejirika, Q. G., Oluchi, N., Nwankudu, N. O., Omeh, N. Y. & Ezeja, I. M. (2018). Antidiarrheal and antioxidant activities of methanol extract of *Bryophyllum pinnatum* leaf harvested from South-Eastern Nigeria in Mice. *Hindawi Journal of Pharmaceutics.* 2018. https:// doi.org/10.1155/2018/6810620

[12] Kendeson, A. C., Kagoro M. L. & Adelakun A. E. (2019). Phytochemical, antioxidant and antimicrobial evaluation of Nigerian *Kalanchoe pinnata* (Lam.), *Journal of Chemical Society of Nigeria*, *44*(6): 1080 - 1085.

[13] Kendeson A. C., Kagoro, M. L. & Adelakun, A. E. (2021). Phytochemical and pharmacological evaluation of Nigerian *Kalanchoe pinnata* (Lam.) Stem-bark. *Journal of Chemical Society of Nigeria*, *46*(4): 0751 - 0756.

[14] Akinpelu, D. A. (2000). Antimicrobial activity of *Bryophyllum pinnatum* leaves. *Fitoterapia*, *71*(2): 193 - 194. https://doi.org/10.1016/S0367-326X(99)00135-5

[15] Igwe, S. A. & Akunyili, D. N. (2005). Analgesic Effects of Aqueous Extracts of the Leaves of *Bryophyllum pinnatum*. *Pharmaceutical Biology*, *43*(8): 658 - 661. https://doi.org/10.1080/13880200500383108

[16] Chaturvedi, Om S., Amit, J. & Bal, K. D. (2012). Pharmacognostical, Phytochemical evaluation and anti-inflammatory activity of stem of *Kalanchoe Pinnata* Pers. *International Journal of Pharmaceutical Science and Research*, *3*(4): 1133 - 1140. http://dx.doi.org/10.13040/IJPSR.0975-8232.3(4).1133-40

[17] Chibli, L. A., Rodrigues, K. C. M., Gasparetto, C. M., Pinto, N. C. C., Fabri, R. L., Scio, E., Alves, M. S., Del-Vechio-Vieira, G. & Sousa, O. V. (2014). Anti-inflammatory effects of *Bryophyllum pinnatum* (Lam.) Oken ethanol extract in acute and chronic cutaneous inflammation. *Journal of Ethnopharmacology*, *154*(2): 330 - 338. http://dx.doi.org/10.1016/j.jep.2014.03.035

[18] Afzal, M., Gupta, G., Kazmi, I., Rahman, M., Afzal, O., Alam, J., Hakeem, K. R., Pravez, M., Gupta, R. & Anwar, F. (2012). Anti-inflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. *Fitoterapia*, *83*(5): 853 - 858. doi:10.1016/j.fitote.2012.03.013

[19] Sofowora, A. (1993). Medicinal Plant and Medicine in Africa, John Willey Spectrum, Ibadan Nigeria; 281 - 285 p.

[20] Harborne, J. B. (1984). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London; 20 - 21 p.

[21] Trease, G. E. & Evans, W. C. (2002). Pharmacognosy. 15th Edition. Churchill Livingstone Harcourt Health services, London, 26, 127, 147 p.

[22] Kaur, C. & Kapoor, H. C. (2002). Antioxidant activity and total phenolic content of some Asian vegetables, *International Journal of Food Science and Technology*, *37*(2): 153 - 161.

https://doi.org/10.1046/j.1365-2621 .2002.00552.x

[23] John, B., Sulaiman, C. T., George, S. & Reddy, V. R. K. (2014). Total phenolics and flavonoids in selected medicinal plants from Kerala, *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(1): 406 - 408.

[24] Sanches, N. R., Garcia, C. D. A., Schiavini, M. S., Nakamura, C. V. & Filho, B. P. D. (2005). An evaluation of antibacterial activities of *Psidium guajava. Brazilian Archives of Biology and technology*, *48*(3): 429 - 436. https://doi.org/10.1590/S1516-8913200 5000 300014.

[25] Ochei, J. & Kolhatkar, A. (2007). Medical Labouratory Science. Theory and Practice. 6th Ed. Tata McGraw-Hill publishing company Ltd; 801 - 807 p.

[26] Okwu, D. E. & Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, *5*(4): 357 - 361.

[27] Majaz, Q. A., Khurshid, M., Nazim, S., Rahil, K. & Siraj, S. (2011) Evaluation of antioxidant activity of *Kalanchoe pinnata* roots. *Int. J. Res. Ayurveda Pharm.*, *2*(6): 1772 - 1775.

[28] Singh, S. K., Patel, J. R. & Dangi, A. (2019). Physicochemical, qualitative and quantitative determination of secondary metabolites and antioxidant potential of *Kalanchoe pinnata* (Lam.) Pers. leaf extracts. *Journal of Drug Delivery and Therapeutics*, *9*(1): 220 - 224.

[29] Mamta, S., Jyoti, S., Rajeev, N., Dharmendra, S. & Abhishek, G. (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*, *1*(6): 168 - 182. http://dx.doi.org/10.22271/phyto

[30] Mir, A. M., Sawhney, S. S. & Jassal, S. M. M. (2013). Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker Journal of Pharmacy and Pharmocology 2*(1): 001 - 005.

[31] Kouitcheu, M. L. B., Eyoum Bille, B., Tchouangueu, T. F., Nguepi, E. & Leundji, H. (2016). Treatment of Helicobacter pylori infected mice with Bryophyllum pinnatum, a medicinal plant with antioxidant and antimicrobial properties, reduces bacterial load. *Pharmaceutical Biology*, *55*(1): 603 - 610.
https://doi.org/10.1080 /13880209 .2016.1266668

[32] Rahman, F., Tabrez, S., Ali, R., Alqahtani, S. A., Ahmed, Z. M. & Rub, A. (2021). Molecular docking analysis of rutin reveals possible inhibition of SARS-CoV-2 vital proteins. *Journal of Traditional and Complementary Medicine*, *11*(2): 173 - 179. https://doi.org/10.1016/j.jtcme.2021.01.006.