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**ASSESSMENT OF ANTIOXIDANT, ANTIMICROBIAL AND ANTI-  
INFLAMMATORY POTENTIAL OF ETHYL ACETATE AND  
ETHANOLIC EXTRACTS OF *Cecropia peltata* L. LEAF EXTRACTS**

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**ABSTRACT**

The present study aims to determine the phytoconstituents and anti-oxidant content, radical scavenging potential, *in vitro* anti-inflammatory and antimicrobial potential of *Cecropia peltata* leaves extracts. The content of phenol, flavonoid, tannin and alkaloids were measured by *in vitro* methods. Scavenging ability of *Cecropia peltata* leaf extracts were assessed against DPPH and hydroxyl radical. The metal chelating potential was also determined. Anti-inflammatory potential of the extracts were determined by albumin denaturation assay. Anti-microbial potential of the ethyl acetate and ethanol extract of *Cecropia peltata* leaves against *Pseudomonas aeruginosa*,

*Pseudomonas vulgaris*, *Escherichia coli*, *Bacillus sp.*, and *Candida albicans* were determined. The results shows that ethyl acetate extract of *Cecropia peltata* leaves possess appreciable amount of phenols, alkaloids and flavonoids with a positive correlation towards free radical scavenging potential of DPPH, hydroxyl radical and metal chelating property.

The IC<sub>50</sub> value of ethyl acetate extract and ethanol extract of *Cecropia peltata* leaves were 494 µg/ml and 493 µg/ml and 542 µg/ml for standard Diclofenac. Among the two, ethyl acetate extract of *Cecropia peltata* leaves inhibited the tested pathogens very effectively than ethanol extract. Thus, the presence of potent antioxidants and antimicrobial property in *Cecropia peltata* leaves will help in drug development against as oxidants and antimicrobial agents.

**Keywords:** *Cecropia peltata*, Anti-oxidant, Antimicrobial, Anti-inflammatory

## INTRODUCTION

The existing wide biodiversity around the world has been left untapped and is waiting for scientist to explore this area for the novel medicines which were available but never been on the market for curing wide range of diseases that are emerging. *Cecropia* sp. are nested in the Urticaceae family. The Urticaceae family has been described as an ordinary plant with extra ordinary functions [1]. The *Cecropia* is a fast growing tree and is found in abundance and wide spread [2] with 6 genera and 170 species. They are lowland species. Phytochemical studies conducted on the vast species of *Cecropia* has showed evidence of secondary metabolites such as tannins, flavonoids, phenolic compounds [3]. Different part of the plant such as the leaf, root, and bark showed effects such as anticancer, anti-inflammatory, anti-diuretic etc. Herbal medicines are found in nature and

are derived from parts of a plant either the leaf, stem, bark or the root. The *Cecropia peltata* found commonly in the forest of Central and South America [4]. It has an interesting feature which is the interaction of the Azteca ants which showcases a classic example of a defense mutualism in the tropics. The indigenous people use different species of this plant as a folk medicine where they take the green leaves and use this to disinfect the genitals prior to child birth. Due to this massive therapeutic potential exhibited by this plant, this study aimed to analyses the antioxidant potential, metal chelating and antimicrobial efficacy of *Cecropia peltata* leaf extracts (ethyl acetate, ethanol).

## MATERIALS AND METHODS

### Plant Collection and Identification

*Cecropia peltata* leaves were collected from the Soesdyke–Linden Highway, Guyana, and

South America in the month of November 2019.

The identification of the plant was done by Ms. Kaslyn-Collins Senior Scientific Officer from the Centre for the Study of Biological Diversity from the University of Guyana, South America.

### **Extract Preparation**

The healthy *Cecropia peltata* leaves were collected from the natural habitats and it was washed with running tap water. It was then left for air-drying under shade then it was ground to a coarse powder. The leaves were extracted by Soxhlet extraction using ethanol and ethyl acetate and the crude extract was stored in an air tight container for further analysis [5].

### **Phytochemical Analysis**

Qualitative tests for alkaloids, flavonoids, steroids, phenols, glycosides, quinones, tannins, carboxylic acid, saponins and coumarin were performed [6]. Determination of total phenol, flavonoid and alkaloid content have been carried out for both the extracts [7].

### **Thin layer chromatography**

The ethyl acetate and ethanol extracts of *Cecropia peltata* leaves (10 µL) were applied on pre-coated TLC plates using capillary tubes and air dried. The TLC plates were developed in a chamber using chloroform: methanol (5:1) as the mobile phase and observed under UV light (254 nm). Caffeic

acid, quercetin, rutin, trans-cinnamic acid and salicylic acid were used as standards. The mobility of the samples was expressed as retention factor (Rf) as calculated using the following formula:  $Rf = \text{Distance travelled by the solute (cm)} / \text{Distance travelled by the solvent (cm)}$  [8].

### **Antioxidant Activity**

The radical 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) was employed to measure both extracts capacity to scavenge free radicals [9]. Free radicals ability to cause mutations is a result of their direct interactions with DNA, which causes DNA damage and is a key factor in the development of cancer [10]. Chelating activity is important in determining an extract's or compound's antioxidant capacity to lower the concentration of metal ions that catalyze lipid peroxidation [11]. In this study antioxidant activity of *Cecropia peltata* leaf extracts were determined by DPPH, hydroxyl radical scavenging and metal chelating activity.

### **Antimicrobial Activity**

Gram positive and gram negative bacterial strains and fungal strain were used in this experiment to know the antimicrobial activity of the sample extracts.

Gram positive bacterial strain *Bacillus subtilis*, gram negative bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*

and *Proteus vulgaris* and fungal strains *Candida albicans* were tested. *In vitro* antibacterial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia. Kirby-Bauer method was followed for disc diffusion assay. The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disc diffusion (Kirby–Bauer) method. *In vitro* antifungal activity was screened by using Potato Dextrose Agar medium and the fungicidal effect of the sample extracts was determined by the inhibition of mycelial growth of the fungus by the extracts which are generally recorded as a zone of inhibition near the wells [12].

#### ***In vitro* Anti-inflammatory Activity**

The AZM (100 mg)-loaded macro emulsion (50 ml) was prepared freshly and 1000–5000  $\mu$ L of emulsion (equivalent to 2000–10,000  $\mu$ g AZM) was mixed individually with 100 ml of pH 7.4 phosphate buffer. From these, 2000  $\mu$ l (equivalent to 40–200  $\mu$ g of AZM) was mixed individually with 200  $\mu$ g of egg albumin powder and 2800  $\mu$ l of pH 7.4 phosphate buffer. Hence, the final concentrations of AZM in each one of the reaction mixtures were ranged from 8 to 48  $\mu$ g/ml. The corresponding control solutions were also prepared using an emulsion without drug and pH 7.4 phosphate buffer. Similarly,

reference standard solutions were made by dissolving 100 mg of Diclofenac sodium in 50 ml of pH 7.4 phosphate buffer and all other steps were also followed to get the final concentration of Diclofenac in each one of the reaction mixtures ranging from 8 to 48  $\mu$ g/ml. All the reaction mixtures were incubated at  $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$  in an incubator (NSW Ltd, New Delhi, India) for 15 min followed by heating to  $70^{\circ}\text{C}$  for 5 min in a water bath. The absorbance for all the reaction mixtures was measured at 660 nm in a spectrophotometer (SHIMADZU UV-1800, Japan). The percentage inhibition of protein denaturation was calculated using the following formula: %inhibition =  $100[V_t/V_c - 1]$  Where,  $V_t$  = absorbance of test sample and  $V_c$  = absorbance of control. Fifty percent inhibition (IC<sub>50</sub>) values were determined for the AZM-loaded emulsion and Diclofenac-containing phosphate buffer solution. The drug concentration for 50% inhibition (IC<sub>50</sub>) was determined by plotting percentage inhibition with respect to control against treatment concentration [13].

#### **RESULTS**

The present study revealed that the presence of flavonoids, phenol, tannins and saponins in the ethanolic extract of *Cecropia peltata* leaves and the alkaloid, flavonoid, phenol, glycoside, tannin, carboxylic acid and

coumarin were found in the ethyl acetate extract (**Table 1**). The variable presence of the secondary metabolites observed in the extracts of *Cecropia peltata* leaves might be due to the difference in the polarity of the solvent used for the extraction.

The ethyl acetate extract of the leaves of *Cecropia peltata* showed highest phenol content with concentration 71.3 µg/ml and the ethanol extract showed 59.1 µg/ml respectively (**Table 2**). Similar trend was observed with the flavonoid and alkaloid content of *Cecropia peltata* leaves. The flavonoid content in ethyl acetate leaf extract was 500 µg/ml and the lowest concentration was found in ethanol extract 403 µg/ml. The ethyl acetate extract showed highest alkaloid content with concentration 401.9 µg/ml and the ethanol extract showed 279 µg/ml (**Table 2**). The presence of antioxidants of different polarity might be the reason for the variable amount of antioxidants observed in ethyl acetate and ethanol extracts of *Cecropia peltata* leaves.

### Free radical scavenging potential

In this study, the 50% DPPH scavenging potential of standard ascorbic acid was found to be  $63.32 \pm 1.62$  µg/ml. The ethyl acetate extract of *Cecropia peltata* leaves scavenged DPPH effectively with at a lower concentration (IC<sub>50</sub> value of  $75 \pm 2$  µg/ml)

when compared with ethanol extract ( $124 \pm 2.6$  µg/ml) (**Figure 1**). The IC<sub>50</sub> value to scavenge hydroxyl radical of standard ascorbic acid was  $39 \pm 1.73$  µg/ml followed by ethyl acetate extract  $101.16 \pm 2$  µg/ml and ethanol extract  $127 \pm 2.08$  µg/ml of *Cecropia peltata* leaves (**Figure 2**).

In the present study the lowest metal chelating impact was seen in the ethanol extract *Cecropia peltata* leaves with an IC<sub>50</sub> value of  $82 \pm 3$  µg/ml and highest by ethyl acetate extract  $50.83 \pm 1.89$  µg/ml (**Figure 3**). Ascorbic acid chelated metal ion at IC<sub>50</sub> value of  $45.67 \pm 2.5$  µg/ml (**Figure 3**).

The results of antimicrobial activity study of the sample extracts of *Cecropia peltata* are presented in **Table 3 and Figure 4**. The ethyl acetate extract showed the highest inhibition against *E. coli* (17 mm) followed by *Proteus vulgaris* (16mm). When compared with the two extracts ethyl acetate showed the highest activity when compared to ethanol extract of the *Cecropia* leaves.

The presence of active substances was examined using TLC. In the current investigation, from the two extracts of *Cecropia* leaves, 6 fractions were observed in ethyl acetate extract and only 4 in ethanol extract (**Table 4**). The R<sub>f</sub> value of the active spots on the TLC-bioautography profile was 0.65mm, and the inhibitory zone's diameter

was 22mm and 26 mm on *E. coli* and *B. subtilis* 26 mm (**Figure 5**). It can be concluded that the bioautography test showed six (6) active spot on the ethyl acetate fraction, which was the most effective fraction at inhibiting the growth of *E.coli*. Active compounds from the chromatogram spot diffused into the media and produced an inhibition zone to develop, which prevented bacterial growth at the active compound's location of diffusion.

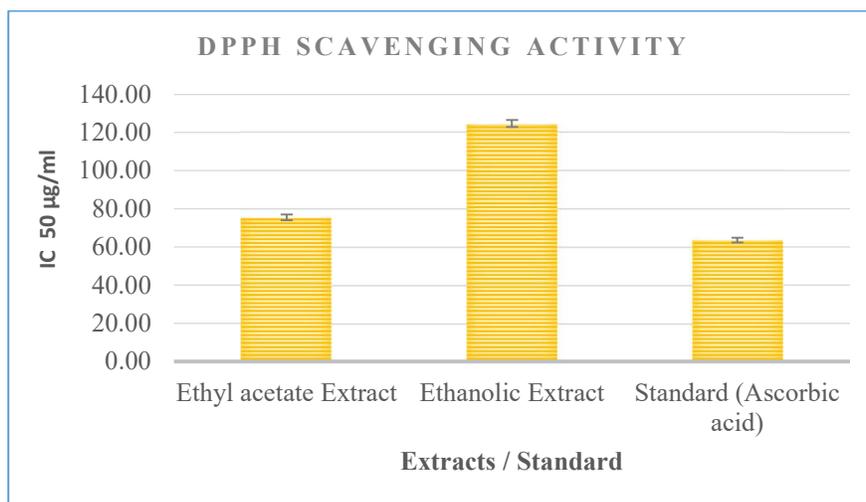
The percentage inhibition values for the protein (albumin) denaturation phenomenon obtained for ethyl acetate extract, ethanol extracts of *Cecropia peltata* leaves and Diclofenac sodium solution used as a positive control is shown in **Table 5**. In this study ethyl acetate extract of *Cecropia peltata* leaves showed an IC<sub>50</sub> value of 494 µg/ml and ethanol showed a 493 µg/ml IC<sub>50</sub> value when compared to IC<sub>50</sub> value of 542 µg/ml by Diclofenac.

**Table 1:** Phytochemical analysis of ethyl acetate and ethanolic extracts of *Cecropia peltata* leaves

Phyto Compounds	Ethyl acetate Extract	Ethanolic Extract
Alkaloids	Present	Absent
Flavonoids	Present	Present
Sterol	Absent	Absent
Phenol	Present	Present
Glycoside	Present	Absent
Quinones	Absent	Absent
Tannins	Present	Present
Carboxylic acid	Present	Absent
Saponins	Absent	Present
Coumarin	Present	Present

**Table 2:** Total phenol, alkaloid and flavonoid content in *Cecropia peltata* leaf ethyl acetate and ethanolic extracts

Extracts of <i>Cecropia peltata</i>	Phenol (µg /gm)	Alkaloid (µg /gm)	Flavonoid (µg /gm)
Ethyl acetate Extract	71.13 ± 1.05	401.93 ± 1.30	503.26 ± 0.73
Ethanolic Extract	58.5 ± 0.55	279 ± 1	403.35 ± 1.52



**Figure 1:** DPPH radical scavenging potential of *Cecropia peltata* leaf ethyl acetate and ethanolic extracts (Results were expressed as Mean ± STD; n=3)

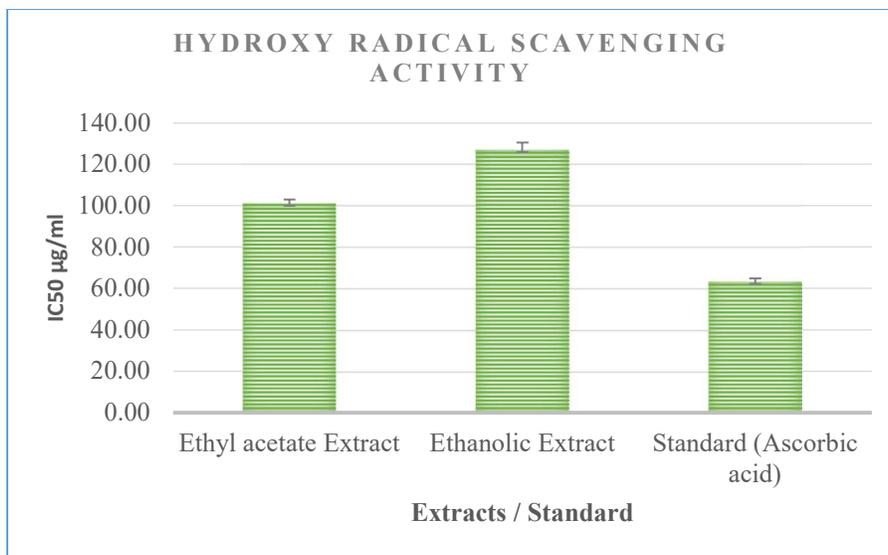


Figure 2: Hydroxyl radical scavenging potential of *Cecropia peltata* leaf ethyl acetate and ethanolic extracts (Results were expressed as Mean  $\pm$  STD; n=3)

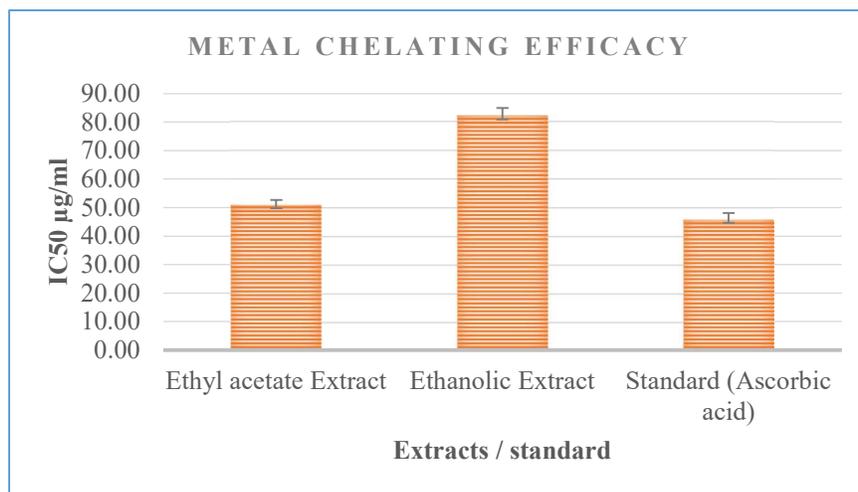


Figure 3: Metal chelating efficacy of *Cecropia peltata* leaf ethyl acetate and ethanolic extracts (Results were expressed as Mean  $\pm$  STD; n=3)

Table 3: Antimicrobial activity *Cecropia peltata* leaf ethyl acetate and ethanolic extracts

Test Pathogens	Et ext	EA extr	NP 25	NP 50	Et	EA	Ofl
<i>P. aeruginosa</i>	8	13	-	16	--	--	16
<i>P. vulgaris</i>	12	16	-	14	--	--	17
<i>E. coli</i>	10	17	-	15	--	--	18
<i>B. subtilis</i>	12	13	-	-	--	--	18
<i>C. albicans</i>	5	15	12	16	--	--	20

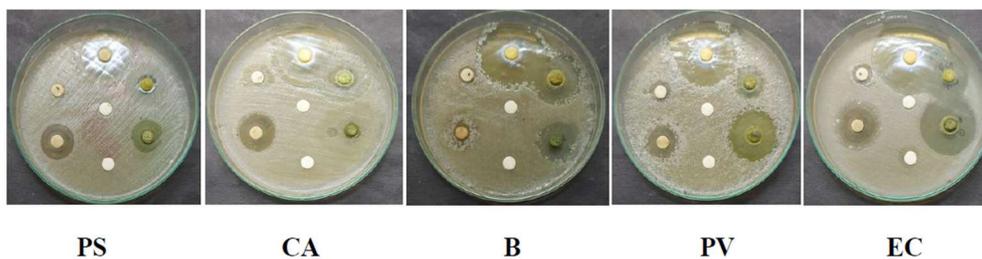
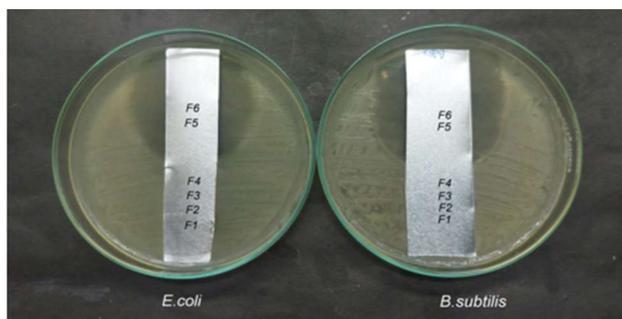


Figure 4: Antimicrobial activity *Cecropia peltata* leaf ethyl acetate and ethanolic extracts

Table 4: Rf vale of fractions

Fraction	Ethyl acetate extract	Ethanol extract
1	0.48	0.51
2	0.54	0.53
3	0.58	0.58
4	0.61	0.61
5	0.63	--
6	0.65	--



Fraction	<i>E. coli</i>	<i>B. subtilis</i>
1	--	--
2	--	--
3	--	--
4	--	--
5	22mm	26mm
6	22mm	26mm

Figure 5: Bioautography of TLC fraction

Table 5: Anti-inflammatory activity of *Cecropia peltata* leaf extracts

Con (µg/ml)	Ethyl acetate	Ethanol	Diclofenac
250	34±0.57	29±1.52	22±0.57
500	52±1.52	38±0.57	38±0.57
750	62±0.57	54±0.57	46±1.52
1000	71±0.57	68±0.57	66±1.52
IC50	494	493	542

## DISCUSSION

Over the years through scientific evidence plants have been reported to have an abundant phytochemical and a wide range of biological properties. Phytochemicals present in every section of the plant suggests that each portion has an equal role in traditional medicine [14]. Many plants phenolic content has been linked to their antioxidant and cytotoxic properties [15]. Nitrogenous chemicals called alkaloids helps to protect plants from infections and herbivores. They are also frequently employed as stimulants, medicines, poisons, and narcotics [16]. The presence of flavonoid,

phenol, tannin, saponins and coumarin has caught the attention of scientist around the world due to the pharmacological effects such as anti-bacterial, antioxidant and anticancer activities [17]. Ethyl acetate extract showed a positive for alkaloid, flavonoid, phenol, glycoside, tannin, carboxylic acid and coumarin all having pharmacological properties including anti-aging and anticancer effects. The presence of the galloyl groups and hydroxyl groups present in the phenols, flavonoids and tannins are responsible for the chemicals chelating and radical-scavenging activities.

## CONCLUSION

*Cecropia peltata* leaves contains a variety of phytochemical compounds. *In vitro* test results indicated that the chemical characteristics of phenolic acids and flavonoids are crucial in supporting the traditional medicinal value of *Cecropia peltata* leaves.

The chelating ability and free radical scavenging potential clearly demonstrate the potential of antioxidants and Inhibition of protein denaturation strongly demonstrated anti-inflammatory potential. This study suggests that the phenolic acids and flavonoids in *Cecropia peltata* contribute to the anti-inflammatory and antioxidant effects.

## CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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## REFERENCES

- [1] Kregiel, D., Pawlikowska, E., & Antolak, H. (2018). *Urtica* spp.: Ordinary plants with extraordinary properties. *Molecules*, 23(7), 1664.
- [2] Rivera-Mondragón, A., Ortíz, O. O., Gupta, M. P., & Caballero-George, C. (2021). Pharmacognostic Evaluation of Ten Species of Medicinal Importance of *Cecropia*: Current Knowledge and Therapeutic Perspectives. *Planta Medica*, 87(10/11), 764-779.
- [3] Hernández Carvajal, J. E., & Luengas Caicedo, P. E. (2013). Estudio fitoquímico preliminar de *Cecropia membranacea* Trécul. *Cecropia metensis* Cuatrec. *Revista Cubana de Plantas Medicinales*, 18(4), 586-595.
- [4] Rivera-Mondragón, A., Bijttebier, S., Tuenter, E., Custers, D., Ortíz, O. O., Pieters, L., & Foubert, K. (2019). Phytochemical characterization and comparative studies of four *Cecropia* species collected in Panama using multivariate data analysis. *Scientific reports*, 9(1), 1763.
- [5] Mueller, S. D., Florentino, D., Ortmann, C. F., Martins, F. A.,

- Danielski, L. G., Michels, M., & Reginatto, F. H. (2016). Anti-inflammatory and antioxidant activities of aqueous extract of *Cecropia glaziovii* leaves. *Journal of ethnopharmacology*, *185*, 255-262.
- [6] Kabuka, R., Mudenda, S., Kampamba, M., Chulu, M., Chimombe, T., & Hikaambo, C. N. A. (2022). Phytochemical Analysis of Leaf, Stem Bark, and Root Extracts of *Cassia abbreviata* Grown in Zambia. *Pharmacology & Pharmacy*, *13*(5), 119-128.
- [7] K Assaf, H., M Nafady, A., E Allam, A., Hamed, A., & S Kamel, M. (2021). Phytochemistry and biological activity of family. *Nafady, Alaa and E. Allam, Ahmed and Hamed, Ashraf and S. Kamel, Mohamed, Phytochemistry and biological activity of family" Urticaceae": a review (1957-2019)(January 31, 2021)*.
- [8] Rizky, H. M., Nanik, S., Nurkhasanah, N., & Ricke, D. (2020). Antibacterial Activity and TLC-Bioautography Analysis of the Active Fractions of *Muntingia calabura* L. leaves against *Staphylococcus aureus*. *Jurnal Farmasi Sains dan Komunitas*, *17*(2), 69-75.
- [9] Fernandes, M. F., Conegundes, J. L. M., Pinto, N. D. C. C., Oliveira, L. G. D., Aguiar, J. A. K. D., Souza-Fagundes, E. M., & Scio, E. (2019). *Cecropia pachystachya* leaves present potential to be used as new ingredient for antiaging dermocosmetics. *Evidence-Based Complementary and Alternative Medicine*, 2019.
- [10] Rajpoot, R., Rani, A., Srivastava, R. K., Pandey, P., & Dubey, R. S. (2018). Protective role of *Mentha arvensis* aqueous extract against manganese induced toxicity by reducing Mn translocation and promoting antioxidative defense in growing *Indica* rice seedlings. *Journal of Crop Science and Biotechnology*, *21*, 353-366.
- [11] Pereira, D. M., Valentão, P., Pereira, J. A., & Andrade, P. B. (2009). Phenolics: From chemistry to biology. *Molecules*, *14*(6), 2202-2211.
- [12] Dahiya, P., & Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical

- isolates. *Indian journal of pharmaceutical sciences*, 74(5), 443.
- [13] Yani, D. F., & Fatahillah, R. Anti-Inflammatory Activity of Ethanol Extract and Ethyl Acetate Fraction of Kebiul (*Caesalpinia bonduc* L.) Seed coat against inhibition of protein denaturation.
- [14] Malik, F., Hussain, S., Sadiq, A., Parveen, G., Wajid, A., Shafat, S., & Raja, F. Y. (2012). Phyto-chemical analysis, anti-allergic and anti-inflammatory activity of *Mentha arvensis* in animals. *Afr J Pharm Pharmacol*, 6(9), 613-619.
- [15] Lacret, R., Varela, R. M., Molinillo, J. M., Nogueiras, C., & Macías, F. A. (2012). Tectonoelins, new norlignans from a bioactive extract of *Tectona grandis*. *Phytochemistry Letters*, 5(2), 382-386.
- [16] Luengas-Caicedo, P. E., Braga, F. C., Brandão, G. C., & Oliveira, A. B. D. (2007). Seasonal and intraspecific variation of flavonoids and proanthocyanidins in *Cecropia glaziovii* Sneth. Leaves from native and cultivated specimens. *Zeitschrift für Naturforschung C*, 62(9-10), 701-709.
- [17] Ri, H. I., Kim, C. S., Pak, U. H., Kang, M. S., & Kim, T. M. (2019). Effect of different polarity solvents on total phenols and flavonoids content, and In-vitro antioxidant properties of flowers extract from *Aurea Helianthus*. *arXiv preprint arXiv:1906.12006*.