Original Article

Antioxidant And Antibacterial Activities Of Important Medicinal Plants Of Family Caesalpiniaceae

Supriya Jayant Janbandhu[1*], Zafar Sikandar Khan[2]

[1]Department of Botany, K.J.Somaiya College of Science and Commerce Vidyavihar, Mumbai 400077 Maharashtra, India.
[2]Department of Botany, KIHSs Maharashtra College of Arts ,Science & Commerce Mumbai 400008 Maharashtra India.

***Corresponding Author -**Supriya Jayant Janbandhu

[*]Department of Botany, K.J.Somaiya College of Science and Commerce Vidyavihar, Mumbai 400077 Maharashtra, India.
Email supriya.j@somaiya.edu
Doi: 10.47750/pnr.2022.13. S05.83

Abstract

Background: Herbal remedies have long been relied upon for the treatment and prevention of illness.

Aim: In this study, the antioxidant and antibacterial efficacy of plant extracts used in traditional medicine was evaluated.
Materials and Methods: Antibacterial activity was assessed using disc diffusion, while antioxidant power was evaluated using the DPPH radical scavenging assay.

Results: Antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and potential antioxidant characteristics were observed in ethanol extracts of *Cassia bonducella, Caesalpinia coriaria, Cassia alata, Brownea coccinea, Cassia siamea, Cassia javanica, Amherstia nobilis,* and *Bauhinia acuminata*.

Conclusion: Traditional uses of these plants to treat conditions related to pathogenic microorganisms and oxidative stress have been confirmed by their promising antibacterial and antioxidant capabilities, which have been used in a wide variety of herbal medicines. To learn more about the medicinal potential of these plants for human use, more research is needed to identify and isolate their active principles, conduct toxicological tests to ensure their safety, and conduct in vivo experiments on appropriate animals.

Key words: Caesalpiniaceae, *Cassia bonducella*, *Escherichia coli*, herbal medicine

INTRODUCTION

Nearly 50,000 people each day lose their lives due to infectious diseases, making them the leading cause of premature death worldwide (Holmes et al., 2017). Human pathogenic germs that are resistant to many drugs have been reported increasingly from around the world in recent years. According to the Centers for Disease Control and Prevention, every year there are around 2 million cases of infections that are resistant to antibiotics (CDC, 2019). The emergence of drug-resistant microorganisms has made it more difficult to treat infectious infections in immunocompromised people including those with AIDS and cancer (Fair and Tor, 2014). The advent of antibiotic-resistant pathogenic microorganisms is one of the gravest public health issues humanity has ever faced.

The body's natural oxidation-reduction system produces singlet oxygen (1O2), superoxide anion (O2), hydroxyl radical (OH), and hydrogen peroxide (H2O2) (Phaniendra et al., 2015). Increased reactive oxygen species production is linked to age-related diseases and disorders (Liguori et al., 2018). Synthetic antioxidants are useful in avoiding or postponing the beginning of many serious illnesses caused by oxidative stress. Many synthetic antioxidants, however, have the drawback and limitation of causing many adverse effects in humans. Since then, the search for natural antioxidants has gained momentum (Sharifi-Rad et al., 2020).

Higher plant antibacterial and antioxidant properties are more common. Eighty percent of the globe relies on conventional medicine for health treatment (Oyebode et al., 2016). Despite WHO's support, promotion, and facilitation of herbal medicine, only 5-15% of the estimated 400,000-500,000 plant species have been rigorously studied for their pharmacological activity (Yuan et al., 2016). A comprehensive study investigated the antibacterial and antioxidant efficacies of many selected Indian medicinal plants to create plant-based therapeutics for the management of diseases caused by pathogenic bacteria and free radicals.

More than 500 species make up the genus Caesalpinia (Caesalpiniaceae), and most of them have not been tested for possible pharmacological action (Baldim et al., 2012). Multiple chemical classes, including diterpenes, flavonoids, and steroids, have been identified from Caesalpinia species (Zanin et al., 2015). Native to India and Malaysia, the thorny and shrubby *Caesalpinia sappan* L. is a small to medium-sized tree that may grow to heights of 4-10 metres and is a member of the family Caesalpiniaceae (Dapson and Bain, 2015). Numerous pharmacological effects, including those that are

antimicrobial, anti-inflammatory, antioxidant, antifungal, anthelmintic, cytotoxic, hepatoprotective, wound-healing, analgesic, anticonvulsant, insecticidal, and antiplasmodial, have been attributed to the plant. *C. bonduc* has been used as a digestive aid, an anthelmintic, an antimalarial, and a therapy for inflammation and poor blood circulation (Pinto et al., 1995; Srinivas et al., 2003; Nagumo et al., 2009). The literature describes a wide variety of chemical constituents and therapeutic significance in the genus Caesalpinia; thus this investigation will focus on the antioxidant and antibacterial capabilities of a subset of those compounds.

MATERIALS AND METHODS

Chemicals and Culture Media

Including Mueller-Hinton agar (MHA), dimethyl sulfoxide (DMSO), β-carotene, hydrogen peroxide (H_2O_2), linoleic acid (LA), butylated hydroxytoluene (BHT), and ascorbic acid were purchased from Hi-media, Mumbai (India). We ordered DPPH from Sigma, an oxidizing agent that is 2,2-diphenyl-1-picrylhydrazyl (Germany). Both the chemicals and solvents utilized were of analytical quality. Natural Substances The study group selected eight different plants (listed in Table 1), all of which are native to the central region of Chhattisgarh and have leaves that are said to have healing capabilities in traditional literature (India).

Collection and extraction of plants

Fresh plants parts were collected from the Veermata Jijabai Bhonsale Udyan, Byculla Mumbai and KJ Somaiya College of Science & Commerce, Mumbai, India. Before being shade dried, crushed, and used in the extraction process, the freshly picked plants were washed twice or three times with standard tap water and once with sterile distilled water. It took 50 grams of powdered plant material and 250 milliliters of 90% ethanol to make the extract. The macerate was filtered using two layers of muslin cloth before being centrifuged at 4000 g for 30 minutes. Filtering the supernatant using Whatman No. 1 filter paper yielded mother extracts, which were then evaluated for antibacterial and antioxidant properties and were considered to be pure (Mohana and Raveesha, 2006).

Evaluation of Antibacterial Activity

The NCIM, National Chemical Laboratory, Pune (India) supplied us with Escherichia coli (NCIM 2065) and Staphylococcus aureus (NCIM 2079), which we subcultured on nutritive agar and stored at 4 degrees Celsius. The bacteria used in the antibacterial activity test were grown in culture for 24 hours. Each plant extract was tested for its antibacterial properties using the cup diffusion method on MHA medium, per the NCCLS-mandated procedure (NCCLS, 2000). To review, a 5-well MHA plate was prepared and inoculated with 100 L of E. coli and S. aureus bacteria (108 CFU/mL) using a sterile wet swab. We next separated 42 plant species into individual wells, added 50 mL of plant extract from each, and incubated the plates at 37°C for 24 hours. The synthetic antibiotic gentamicin (10 µg/disc) was used as a positive control with an identical volume of ethanol. A millimeter-scale measurement of the well-perimeter zone of inhibition (ZOI) was performed (mm). There were four sets of data kept for each therapy. Plants exhibiting Zones of inhibition (ZOI) > 8.0 against Staphylococcus aureus and > 6.5 against Escherichia coli were selected for further testing of their antibacterial activities.

Evaluation of Antioxidant Activity

2, 2-diphenyl-1-picrylhydrazyl Radical Scavenging Assay

A modified version of Ebrahimabadi et al., 2010 method was used to test nine plant extracts for antibacterial and DPPH radical scavenging capabilities (2010). Extracts were combined with 3 mL of DPPH solution (40 mg/L in methanol) and incubated at room temperature and in the dark for 30 minutes. Incubated solutions were analyzed by a UV-VIS spectrophotometer for their absorbance at 517 nm. Negative controls included a methanol solution of DPPH, whereas positive controls included ascorbic acid and BHT at the same concentration. Matching plant extracts were used, and a similar quantity of extracts devoid of DPPH were used as negative controls. Following this formula, we were able to determine the percentage of DPPH radical inhibition:

$$I\% = \frac{(A\ control - A\ sample)}{(A\ control)} \times 100$$

Where $A_{control}$ and A_{sample} are the absorbances of the control and test samples, respectively.

Statistical Analysis

Averages and standard deviations were used to report numerical data. Using GraphPad prism version 5.0 software, we performed an analysis of variance on the data to identify statistically significant differences between groups, and then used Tukey's multiple comparison tests to narrow down the field. When comparing results, differences at the * $P < 0.05$ level were considered significant.

RESULT AND DISCUSSION

Eight plant extracts were evaluated at 0.01, 0.03, 0.06, 0.12, 0.25, and 0.5 ppm for their capacity to kill S. aureus and E. coli, Gram-positive and Gram-negative bacteria. Millimeter-scale measurements of the ZOI diameter around the well are shown in Table 1. All eight plants studied (Cassia bonducella, Caesalpinia coriaria, Cassia alata, Brownea coccinea, Cassia siamea, Cassia javanica, Amherstia nobilis, and Bauhinia acuminata) showed antibacterial action against S. aureus when

their ethanolic extracts were applied to the bacteria. However, highest ZOI was observed for C. bonducella i.e. 31 mm (Figure 1) and 24 mm (Figure 2) for *S. aureus* and *E. coli*, respectively. Interestingly, C. bonducella showed highest MIC against both *S. aureus* (1.43) and *E. coli* (2.03), in comparison to the control group (Table 1). Figure 3 and Figure 4 showing culture plates for *S. aureus* and *E. coli*.

C. sappan ethanolic extract has been shown to be the most effective in terms of providing biological activity, according to the literature (Hemalatha et al., 2007). Both Bacillus subtilis and Chromobacterium violaceum were susceptible to isobonduceline, another compound isolated from the same plant (Srinivas et al., 2003). By employing the diffusion technique to measure the zone of inhibition and the minimum inhibitory concentration, we examined the potential antibacterial effects of a methanolic extract of C. bonducella seeds on Gram-positive and Gram-negative bacteria (MIC). In terms of antibacterial efficacy, this extract was shown to be on par with the reference standard, kanamycin. The triterpenoids alpha-amyrin, alpha-amyrin and lupeol and lupeol acetate were isolated as the active components after fractionation (Saeed and Sabir, 2001).

A methanol extract of C. sappan stems was used to evaluate the bacteria in the intestines. Clostridium perfringens growth was greatly decreased by the most effective component, 5-hydroxy-1,4-naphthoquinone, at 5.0 and 2.0 mg/disc, and moderately at 1.0, 0.5, and 0.25 mg/disc. Furthermore, at both 5.0 and 2.0 mg/disc, this drug had a negligible influence on the proliferation of Lactobacillus casei. Similar naphthoquinones, including 5-hydroxy-2-methyl-1,4-naphthoquinone, showed only moderate suppression against C. perfringens at both 5.0 and 2.0 mg/disc, in contrast to 1,4-naphthoquinone, which substantially inhibited the growth of all bacteria tested at this concentration.

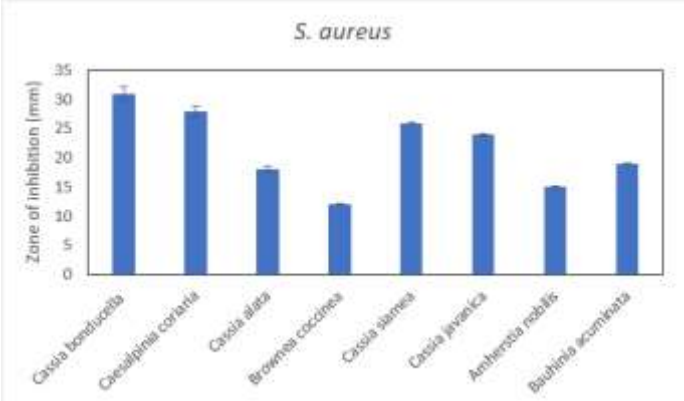

Figure 1 Antibacterial activity of plant extracts against *S. aureus*

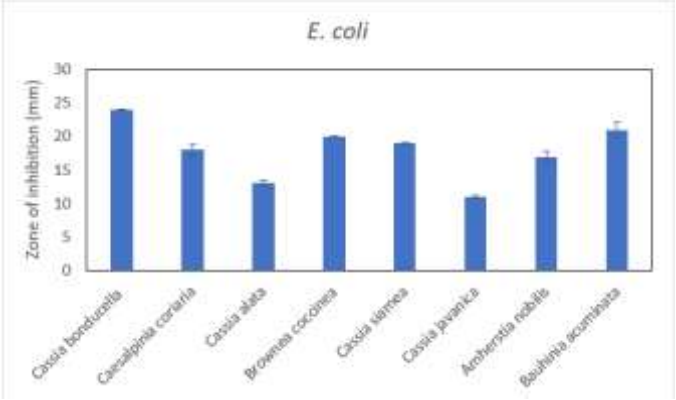

Figure 2 Antibacterial activity of plant extracts against *E. coli*

Table 1 Minimum inhibitory concentration of selected plants of family Caesalpiniaceae

S. No.	Plant extracts/Drug	S. aureus (µg/ml)	E. coli(µg/ml)
1	Control	-	-
2	*Cassia bonducella*	1.43 *	2.03 *
3	*Caesalpinia coriaria*	2.34 *	2.56
4	*Cassia alata*	3.25	3.49
5	*Brownea coccinea*	5.34	2.10 *
6	*Cassia siamea*	2.46 *	2.23
7	*Cassia javanica*	3.01	4.20
8	*Amherstia nobilis*	3.21	3.20
9	*Bauhinia acuminata*	4.11	2.08 *
10	Gentamicin	0.34 *	0.67 *

Data are represented as mean ± SD (n=3), significantly different in comparison to control group.

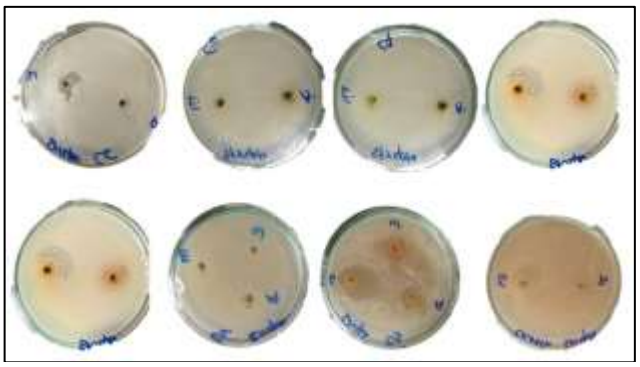

Figure 3 Culture plates of treated plant extracts against *S. aureus*

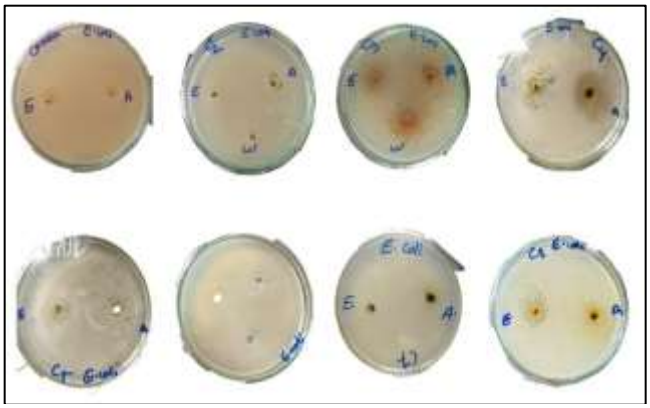

Figure 4 Culture plates of treated plant extracts against *E. coli*

Antioxidant activity

Several plant extracts were tested for their antioxidant activity by analysing their ability to quench DPPH radicals. Diverse samples' scavenging capacities have been extensively examined with the free radical compound DPPH (Kusuma et al., 2014). A sensitive antioxidant assay, the diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test requires hydrogen donation and/or radical scavenging action. The ability of plant extracts to scavenge free radicals is commonly assessed by their hydrogen atom or electron donation ability independent of any enzyme activity, even though the DPPH radical is of relatively little concern to biological systems and living organisms (Mileva et al., 2014).

Measurements of an antioxidant's ability to scavenge the DPPH radical are preferred over those of the hydroxyl or superoxide radicals because of the DPPH radical's greater stability. The DPPH test typically comprises a hydrogen atom transfer process (Li et al., 2009). When antioxidant molecules react with the radical, they scavenge it by donating hydrogen atoms, causing the absorption of DPPH to drop. This is represented conceptually as a transition from purple to yellow.

Measurements of an antioxidant's ability to scavenge the DPPH radical are preferred over those of the hydroxyl or superoxide radicals because of the DPPH radical's greater stability.

The activity of other plant was in the order *Cassia siamea*(8.39±1.39µg/ml)>*Caesalpinia coriaria*(9.29±0.79µg/ml) >*Cassia alata*(14.49±1.08µg/ml) >*Cassia javanica*(16.27±1.38µg/ml) >*Bauhinia acuminata*(19.38±1.13µg/ml) >*Brownea coccinea*(21.33±1.13µg/ml).

Table 2 DPPH radical scavenging activity of selected plants of familyCaesalpiniaceae

S. No.	Plant Extract	IC_{50} (µg/ml)
1	*Cassia bonducella*	5.39±0.19
2	*Caesalpinia coriaria*	9.29±0.79
3	*Cassia alata*	14.49±1.08
4	*Brownea coccinea*	21.33±1.13
5	*Cassia siamea*	8.39±1.39
6	*Cassia javanica*	16.27±1.38
7	*Amherstia nobilis*	23.28±1.81
8	*Bauhinia acuminata*	19.38±1.13

Data are represented are mean ± SD (n=3)

In most cases, it is impossible to pinpoint the actions of individual compounds within plant extracts that are responsible for their biological effects. It's possible that a variety of chemicals working together accounts for the scavenging effect (Jovtchev et al., 2018). To what extent total phenolic content correlates with antioxidant activity in plant products has

been the subject of several research. Antioxidants and radical scavengers are substances that can scavenge DPPH. Enhanced DPPH scavenging activity is associated with increased total phenol and flavonoid levels (Lefahal et al., 2018). It was hypothesised by Lee and coworkers that chemicals isolated from C. sappan's stem could be effective as prophylactics against C. perfringens infection (Lee et al., 2007). Ethanolic extract of C. bonducella seeds showed better free radical scavenging activity than ascorbic acid (AA, IC50 = 26.68 µg/mL) in a 2,2-diphenyl-1-picrylhydrazyl (DPPH) experiment. Again, the extract was not as powerful as AA (IC50 = 70.79 µg/mL) in a hydroxyl radical activity test, but it was nonetheless effective. The extract and AA both showed similar anti-radical properties in an activity assay measuring NO radical scavenging, with IC50 values of 102.65 and 65.98 µg/mL, respectively. The extract has an IC50 of 89.84 µg/mL against the superoxide radical, while the IC50 for AA was 36.38 µg/mL. (Shukla et al., 2009).
C. sappan heartwood ethanolic extract (CSE) and its separated constituents were investigated for in vitro antioxidant activity using the MDA assay. Vitamin C blocked 14.02 percent of the radical at 0.368 µg/mL, but the CSE did so at 0.0006 µg/mL, making the CSE 600-fold more effective. Compounds 35 and 36 had the largest quantities, 20.3% (0.147 µg/mL) and 19.5% (0.102 µg/mL), showing a synergistic impact with CSE. Compound 23 was at 0.54% (0.074 µg/mL) concentration.

CONCLUSION

Scientists are looking for alternatives in natural sources like plants due to the rise of multidrug-resistant human pathogenic microorganisms and the unpleasant side effects of these medications. Previously, it was hypothesized that plants *like Cassia bonducella, Caesalpinia coriaria, Cassia alata, Brownea coccinea, Cassia siamea, Cassia javanica, Amherstia nobilis,* and *Bauhinia acuminata* possessed antibacterial and antioxidant properties, and the current study validates this. The research backs up the conventional wisdom that these plants have curative properties for a wide range of illnesses.

REFERENCES

1. Baldim J, Carvalho B, Martineli P, Santos M, Sartorelli P, Viegas Junior C, et al. The Genus Caesalpinia L. (Caesalpiniaceae): Phytochemical and Pharmacological Characteristics. Molecules. 2012;17:7887–902.
2. CDC. AR Threats Report: Antibiotic Resistance Threats in the United States, 2019. https://www.cdc.gov/drugresistance/biggest-threats.html
3. Dapson R, Bain C. Brazilwood, sappanwood, brazilin and the red dye brazilein: From textile dyeing and folk medicine to biological staining and musical instruments. Biotech Histochem.2015 Apr 20;90:1–23.
4. Ebrahimabadi AH, Djafari-Bidgoli Z, Mazoochi A, Kashi FJ, Batooli H. Essential oils composition, antioxidant and antimicrobial activity of the leaves and flowers of Chaerophyllummacropodum Boiss. Food Control 2010;21:1173-8.
5. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. PerspectMedicin Chem. 2014;6:25-64.
6. Flavanoids from Caesalpinia pulcherrima. Phytochemistry 2003, 63, 789–793.
7. Hemalatha, K.; Kiran, A.S.; Bannappa, U.; Satyanarayana, D. Analgesic activity ofCaesalpinia sappan heartwood. Pharm. Biol. 2007, 45, 360–362.
8. Holmes KK, Bertozzi S, Bloom BR, et al. Major Infectious Diseases: Key Messages from Disease Control Priorities, Third Edition. In: Holmes KK, Bertozzi S, Bloom BR, et al., editors. Major Infectious Diseases. 3rd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2017 Nov 3. Chapter 1.
9. Hong, C.H.; Hur, S.K.; Oh, O.J.; Kim, S.S.; Nam, K.A.; Lee, S.K. Evaluation of natural productson inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in culturedmouse macrophage cells. J. Ethnopharmacol. 2002, 83, 153–159.
10. Lee, H.; Lim, M.; Jeon, J.; Jeong, E.; Lee, C. Antimicrobial activity of 5-hidroxy-1,4-naphtoquinine isolated from Caesalpinia sappan toward intestinal bacteria. Food Chem. 2007,100, 1254–1258.
11. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018 Apr 26;13:757-772.
12. Mohana DC, Raveesha KA. Anti-bacterial activity of Caesalpinia coriaria (Jacq.) Willd. against plant pathogenic Xanthomonas pathovars: An eco-friendly approach. JAgricTechnol 2006;2:317-27.
13. Nagumo, S.; Whasiyama, M.; Sasaki, Y.; Hosokawa, T. Anti-inflammatory constituents of
14. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Disk Susceptibility tests. Approved Standard, 7th ed.. Wayne, Pa: NCCLS Document M2-A7, NCCLS; 2000.
15. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 2016 Oct;31(8):984-91.
16. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015 Jan;30(1):11-26.
17. phenolic content of ethanolic extract of Caesalpinia bonducella seeds. Food Chem. Toxicol. 2009,47, 1848–1851.
18. Pinto, A.C. O Brasil dos viajantes e exploradores e a Química de ProdutosNaturaisBrasileira.Quim Nova 1995, 18, 608–615.
19. Saeed, M.A.; Sabir, A.W. Antibacterial activity of Caesalpinia bonducella seeds. Fitoterapia. 2001, 72, 807–809.
20. Sappan lignum. Biol. Pharm. Bull. 2009, 32, 941–944.
21. Sharifi-Rad M, Anil Kumar N V, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Frontiers in Physiology. 2020; 11:6-12.
22. Shukla, S.; Mehta, A.; John, J.; Singh, S.; Mehta, P.; Vyas, S.P. Antioxidant activity and total
23. Srinivas, K.V.N.S.; Rao, Y.K.; Das, I.M.B.; Krishna, K.V.S.R.; Kishore, K.H.; Murty, U.S.N.Flavanoids from Caesalpinia pulcherrima. Phytochemistry 2003, 63, 789–793.
24. Srinivas, K.V.N.S.; Rao, Y.K.; Das, I.M.B.; Krishna, K.V.S.R.; Kishore, K.H.; Murty, U.S.N.
25. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5).
26. Zanin, João L. B. et al. Caesalpinioflavone, a New Cytotoxic Biflavonoid Isolated from Caesalpinia pluviosa var. peltophoroides. Journal of the Brazilian Chemical Society. 2015;26(4): 804-809.
27. Kusuma IW, Arung ET, Kim YU. Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Sci Hum Wellness. 2014;3:191–196.
28. Mileva M, Kusovski V, Krastev D, et al. Chemical composition, in vitro antiradical and antimicrobial activities of Bulgarian Rosa alba L. essential oil against some oral pathogens. Int J CurrMicrobiolAppl Sci. 2014;3:11–20.
29. Li W, Hosseinian FS, Tsopmo A, et al. Evaluation of antioxidant capacity and aroma quality of breast milk. Nutrition. 2009;25:105–114.
30. Jovtchev G, Stankov A, Georgieva A, et al. Cytotoxic and genotoxic potential of Bulgarian Rosa alba L. essential oil – in vitro model study. BiotechnolBiotechnol Equip. 2018;32:513–519.

31. Lefahal M, Zaabat N, Ayad R, et al. Contents, antioxidant and photoprotective activities of crude methanolic extract of aerial parts of Capnophyllumperegrinum (L.) lange (Apiaceae) growing in Algeria. Medicines. 2018;5:1–10.