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Evaluation of the synergistic potential of *Camellia sinensis* and Badri Cow urine through UV-shielding assay and estimation of antioxidant and antibiofilm activity

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Abstract

The uses of plant and animal-derived products as medicines have long record to treat and manage different types of diseases. The combination of *Camellia sinensis (C. Sinensis)* and cow urine is never been investigated in regards to its sun protection factor (SPF), antioxidant and antibiofilm activities. The combined activity of both extracts showed potent photoprotective activity (SPF value-33.75±0.25), and antioxidant activity with free radical inhibition was 73.94%. Natural products can avertible oxidative stress and act as antioxidants that are used as potent sunscreen. According to the Congo red assay, *Escherichia coli*, *Klebsiella pneumoniae*, and *Listeria monocytogenes* displayed robust biofilm formation capabilities, while *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* exhibited moderate biofilm formation tendencies. *Candida albicans*, on the other hand, showed a weak tendency for biofilm production. The combined extract demonstrated significant antibiofilm effects, particularly potent against Listeria monocytogenes (96.28%), followed by *Klebsiella pneumoniae* (79.62%) and *Escherichia coli* (76.91%). It is concluded that combined formulation could be used as an alternative remedy against to biofilm formation to avoid the various adverse effects of synthetic drugs.

Keywords: *Camellia sinensis*, Cow urine, Photoprotective activity, DPPH, Congo red assay, UV radiation.

INTRODUCTION

A biofilm represents a community of certain microbial cells adhered to a surface, enveloped in exopolysaccharides, and is recognized as a significant source of various challenges, especially in domains like the food processing industry, healthcare, and everyday trade [1,2.3]. In food preparing environments, the availability of biofilms poses a considerable risk of contamination, potentially leading to food spoilage and adulteration. Bacterial biofilms exhibit higher resistance to antibiotics compared to individual planktonic cells [4]. Key components such as polysaccharides, nucleic acids, proteins, phospholipids, and humic substances play pivotal roles in biofilm formation. Approximately 75-89% of the biofilm's exopolysaccharides are responsible for surface adherence. Previous research and finding has demonstrated the effective property of Phyto extracts in preventing biofilm formation and adhesion [5,6,7].

Ultraviolet (UV) radiations cause harmful effects on human skin. It exists between X-rays and visible rays (40-400 nm) and is classified into UV-C (200-280 nm), UV-B (280-320 nm) and UV-A (320-400 nm). UV rays cause skin aging, mutagenicity, immune depression and cancer [8]. From the biological perspective, UV-C is the most harmful radiation filtered by the protective ozone layer. while UV-B is considered to be less harmful to the skin as it can harm skin without causing skin cancer [9]. UV radiation has two opposing effects on the skin: direct effects that cause tanning and sunburn. There are two types of antagonistic impact of UV radiation within the skin: immediate effects that cause tanning and sunburn while delayed-type effects cause cancer and aging [10]. As the skin is exposed to UV radiation, it causes an alteration in collagen, elastin and connective tissues, responsible for skin elasticity and firmness [11].

Oxidative damage affects the total protein, and enzyme and reduces ascorbic acid levels in the cells. The free radicals production in the body is mainly because of oxidation reactions. The body’s antioxidant system can prevent oxidative stress, which often occurs after physiological processes [12]. The pollutants, physical and dietary stress leads to generate free radicals inside the body, therefore, to neutralize these free radicals the dietary antioxidant can contribute to enhancing the antioxidant system by thwarting lipid peroxidation and restraining the onset of chronic diseases. [13],[14]. Therefore,this study aimed to assess the therapeutic efficacy of cow urine extract of *C. sinensis* as both products have long been used as medicinal drinks against various ailments, particularly in India and some other regions of the world.

MATERIAL AND METHOD

Collection of *Badri* cow urine and Tea leaves

Fresh young leaves of Camellia sinensis L. were gathered from the Tea Estate in Dehradun, Uttarakhand, India. Urine from Badri cows, an indigenous breed of Uttarakhand, was collected from the Cattle Breed Centre in Nariyalgaun, Champawat, Uttarakhand. The urine sample was collected mid-stream in a sterile container with meticulous sterile precautions. Subsequently, the Badri cow urine was filtered using Whatman No.1 filter paper to eliminate impurities and then subjected to distillation through a distillation unit to yield cow urine distillate (CUD).

Extraction

Cow urine distillate (CUD) and distilled water (DW) were used as a solvent duringthe extraction process. Leaves of *C. sinensis* were properly washed to avoid dust particles followed by shade-dried and powdered with the help of a grinder.Homogenized the dry powder (5g) of *C. sinensis*with 50 ml cow urine distillate and distilled water separately and abbreviated as CSCUD and CSDW, respectively. Homogenates were then filtered through filter paper.

Screening of biofilm-producing bacteria

Bacterial strains: Six bacterial strains, namely Staphylococcus aureus (MTCC 7443), Pseudomonas aeruginosa (MTCC 424), Escherichia coli (MTCC 118), Salmonella typhi (MTCC 733), Listeria monocytogenes (MTCC 657), and Klebsiella pneumoniae (MTCC 432), were acquired from the Microbial Type Culture Collection Center (MTCC) in Chandigarh, India. The viability of these bacterial strains was assessed by inoculating them onto nutrient broth and then incubating them at 37°C. Stock cultures were maintained through periodic sub-culturing.

Congo red agar method

Bacterial biofilm production was assessed by the Congo red agar (CRA) method. Congo red a gar medium contains 37 g/L brain infusion (BHI) agar mixed with 36 g/L sucrose and 0.8 g C ongo red (dye) and then autoclaved at 121 °C for 15 min. Agar plates were inoculated with th e corresponding bacteria and cultured at 37°C. Based on colony characteristics, bacterial strains were categorized as strong biofilm producers (black colonies), and moderate biofilm producers (red colonies) on the BHI agar medium [15].

Evaluation of antibiofilm activity by crystal violet assay (CVA)

To assess the antibiofilm properties of CSCUD and CSDW, we utilized the Crystal Violet Assay (CVA) specifically targeting bacterial strains known for robust biofilm formation. The methodology involved dispensing 100 µl of the test sample into microtiter plate wells, followed by adding an equal volume of freshly prepared bacterial suspension. The plate was then placed in a 37°C incubator for 48 hours. Following incubation, the contents of each well were gently removed via tapping, and the wells were rinsed with 200 µl of sterile saline solution to eliminate free-floating bacteria. The adherent cells within the wells were stained with 0.1% crystal violet and allowed to incubate at room temperature for 20 minutes. Afterward, the wells were washed with deionized water, and 200 µl of 96% ethanol was added to the plate. The optical density (OD) of the stained adherent cells was measured at 630 nm using an ELISA reader. To ensure precision and reproducibility, all experiments were conducted in triplicate [16].

$$[(OD_{growth\ control} - OD_{sample}) / OD_{growth\ control}] \times 100$$

Estimation of Sun Protection Factor (SPF)

The extracted material (CSCUD and CSDW) was centrifuged at 5000 rpm for 10 min. Supernatants were taken and volume made up to 50 ml by adding methanol, shaken properly and optical density (OD) was measured at 290-320 nm at 5 nm intervals. The efficacy of sunscreen is expressed by using the formula developed by Mansur et al. (1986) [17]. Being an easy, effective formula, it can be a reliable and cost-effective method to determine the sun protection factor (SPF) *in-vitro*. It is expressed by SPF utilizing Spectrophotometry with the following equation:

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where, CF = correction factor (10)

EE (λ) = Erythemal effect of radiation with wavelength λ

I (λ) = Solar intensity spectrum

Abs (λ) = Spectrophotometric absorbance values at wavelength λ

The values of EE x λ are constant (Table 1) (Sayre et al., 1979) [18]

Free radical scavenging activity by the DPPH method

The antioxidant activity of both the extracts (CSCUD and CSDW) was carried out by DPPH assay as per the procedure given by Adhikari et al. [19].

Statistical analysis

The trials were performed independently on three occasions, and the outcomes were expressed as the mean values along with the standard error. Microsoft Excel 2016 was utilized for conducting analysis of variance (ANOVA) to evaluate the data. Significance was determined at a level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Antibiofilm Activity

Biofilm is an aggregation of microbial populations that is highly impermeable to antimicrobial drugs. The emergenceof biofilm leads to innumerable difficulties in the pharmaceutical, food industry and health care settings. Therefore, the novel approachescould play a crucialrole to control biofilm formation and its development. In the present study, out of the seven microbial strains, *E. coli*, *K. pneumonia*e and *L. monocytogenes* showed black colonies i.e. strong biofilm producers followed by *S. typhi*, *S. aureus* and *P. aeruginosa* showed moderate biofilm-producing ability. Meanwhile, *C. albicans* showed pink colonies and were considered to be weak biofilm producers.

In this study, the anti-biofilm efficacy of CSCUD and CSDW was assessed against three specific bacterial strains namely, *L. monocytogenes*, *K.pneumoniae* and *E.coli* by microtiter plate assay. Both the extracts showed varied inhibitory effectsagainstselected biofilm-producing bacterial strains in a dose-dependent manner.Meanwhile, CSCUD showed a significant inhibitory effect against *L. monocytogenes* followed by *K. pneumoniae* and *E.coli.* Medicinal trees are counted to be a potential source of antimicrobial compounds and are also reportedto haveanti-biofilm activity against deleterious microbes such as *E. coli, Salmonella* spp, *P. mirabilis, K. pneumoniae* [20,21].

This outcome shows the efficacy of herbal products as potent antimicrobial potential. The antimicrobial activity of *C. sinensis* and cow urine was reported earlier by Archana and Abraham, [22] and Ahuja *et al* [23] respectively. However, the combination of these two medicinal drinkshas not been much explored and therefore, extensive research on such

combinations may pave a new way, particularly in the field of medicine. Products derived from herbal preparation have experienced great growth in the pharmaceutical industry in the last few years, but these industries have developed many new drugs and pesticides to protect the immune system [24]. Products of plant and animal origin are very useful products that contribute to human health. Cow's milk has been used by rural people since ancient times as an antiseptic against infections and dermatitis. The use of cow urine and plant extracts is very important in the treatment of diseases, especially microbial diseases.

Catechins present in *C. sinensis* assist the damage in the bacterial membrane and bind to the bacterial bilayer [25]. The ability of plant extracts to readily inhibit cell attachment to surface than to inhibit the growth of established biofilms [26]. Phenolic compounds are also found in cow urine and contain hydroxyl group. These hydroxyl groups kill Gram positive and Gram-negative bacteria [27]. Synergistically, *C. sinensis* and cow urine distillate (CSCUD) showed significant inhibitory activity as compared to distilled water extract of *C. sinensis* (CSDW) (Table: 1 and 2).

Table 1: Screening of biofilm-producing bacteria.

Bacteria	Colony Colour	Biofilm Production
Escherichia coli	Black	Strong
Salmonella typhi	Red	Moderate
Klebsiella pneumoniae	Black	Strong
Listeria monocytogenes	Black	Strong
Candida albicans	Pink	Weak
Pseudomonas aeruginosa	Red	Moderate
Staphylococcus aureus	Red	Moderate

Table 2: Percentage inhibition of biofilm-producing bacteria.

Bacteria	CSCUD	CSDW
L. monocytogenes	94.14	79.97

K. pneumoniae	71.23	66.71
E. coli	69.98	69.46
Abbreviation: CSCUD: *C. sinensis* cow urine distillate; CSDW: *C. sinensis* distilled water.		

The *in vitro*, SPF testing is a quantitative method used to measure the efficiency of products used to prevent skin damage and sunburn due to harmful UV radiation. The SPF value of CSCUD and CSDW was 18.65 and 13.84, respectively (Table: 3 and Figure: 1). The formulation used in the present study was found more effective as compared to previously reported plantsviz., *Mentha piperita* (8.184), *Azadiracta indica* (4.368), *Oscimum sanctum*(2.904), *Aloe vera* (5.437), *Lycopersicon esculantum* (6.083), *Carica papaya* (2.310) [28]. Therefore,the combination of plant extract and cow urine distillate could be used to protect against skin allergies caused by UV rays. Earlier, it has been reported that the *Camellia* species possess a photoprotective formulation that prevents skin damage [29] and erythema and edema caused by UV-B radiation.

Table 3: EE × I (constant value) used in the calculation of SPF.

Wavelength (nm)	EE× I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

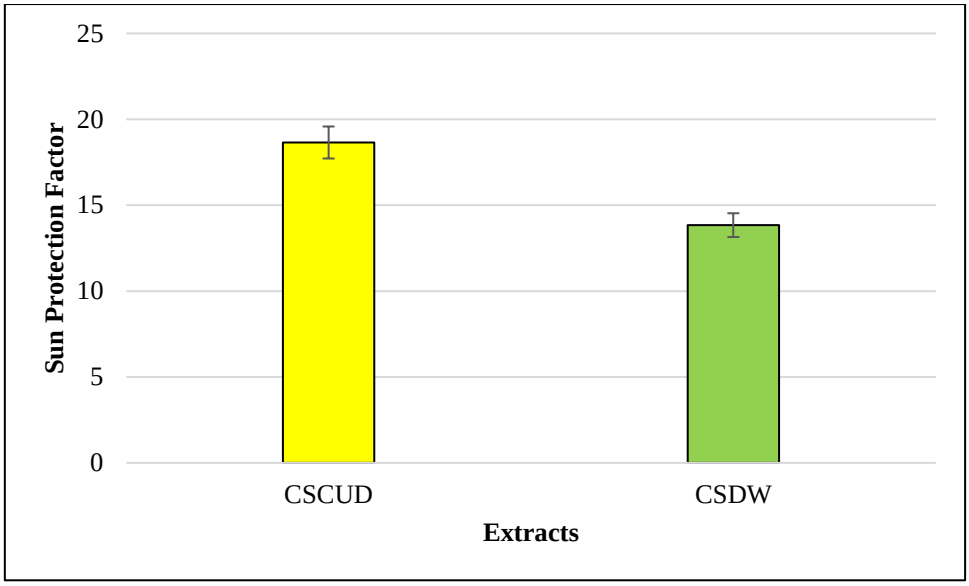

Figure 1: **Sun Protection Factor of CSCUD AND CSDW (P≤ 0.05).**

Antioxidant Activity

Both herbal plants and cow urine are recognized as abundant sources of natural antioxidant compounds [30,31]. Numerous plant species are utilized in traditional medicine for treating various ailments owing to their phytochemical constituents, which possess significant medicinal properties [32]. In this investigation, we assessed the antioxidant potential of CSCUD and CSDW through the DPPH free radical scavenging assay.

The dose-dependent scavenging activity of both the extractwas observed and illustrated in Figure: 2. The previous study done by Lavania et al. [33] reported the free radical scavenging capacityof cow urine. Further, *Panchgavya* (Ayurvedic formulation) that contains cow urine as one of the essential ingredients showed 14.42 % scavenging at 200 µg concentration [34]. Meanwhile, cow urine distillate used as solvent with *C. sinensis* showed potent antioxidant activity. It was reported in the past that UV-B rays absorbed by the skin caused the formation of vigorous free radicals such as (O_2, $^1O_2$, HO_2, OH, ROO) [35]. Hence, the compounds having antioxidants potential are integrated to make sunscreens and are now widely recommended for protection from UVrays. In this context, it has also has been reported that the evaluating the antioxidant properties of the best outcomes is important for the development ofeffective sunscreens [36]. To our knowledge, no research paper on this work have been published to date especially *C. sinensis* extract with Badri cow urine.

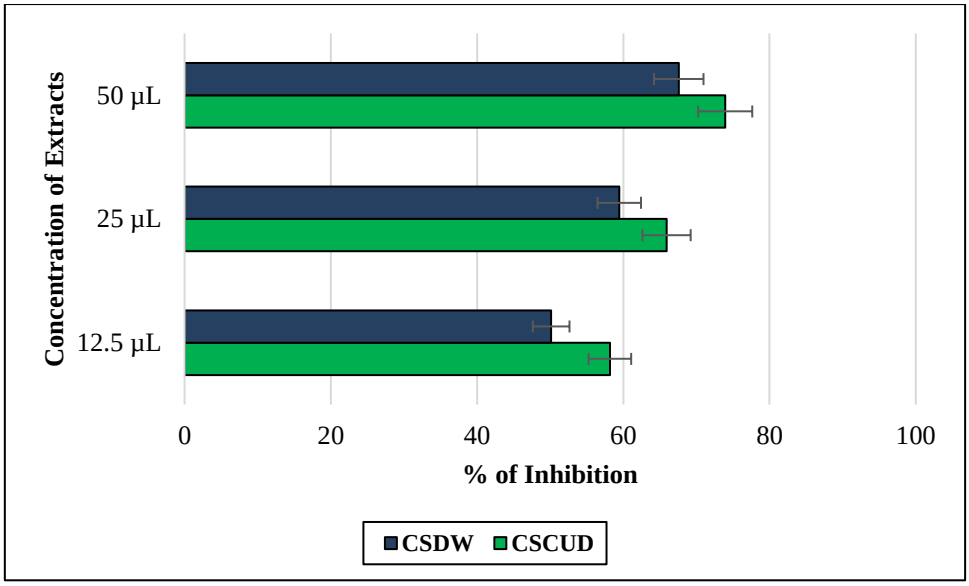

Figure 2: Antioxidant activity of CSCD and CSDW by DPPH assay (P≤ 0.05).

The antioxidant potential of the *Camellia* spp. is already affirmed in previous work done by Selka et al. (2023). Similarly, cow urine demonstrated effectiveness against free radicals generated within the reaction mixture. This efficacy can be attributed to components present in cow urine such as creatinine, calcium, phenols, aurum hydroxide carbolic acid, manganese, and creatinine, all of which may exhibit antioxidant properties [38]. Therefore, the synergistic action of these components could offer enhanced effectiveness against various diseases. By neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism, they protect essential cell components [39]. Free radical reactions are significant pathways in various biological systems, as evidenced by studies across unrelated biological contexts. The study revealed that the antioxidant potential of Camellia sinensis and Badri cow urine distillate may hold therapeutic promise against oxidative threats to health and diseases. The findings underscored that the antioxidant capacity observed is primarily due to the free radical scavenging activity of CSCUD, which could potentially impede the aging process and combat life-threatening diseases.

CONCLUSION

It may be concluded that the use of combined therapy can broaden the inhibition spectrum against UV, free radicals, and biofilm-producing microbes. The combination of potential bioactive compounds from two sources could be significant in different aspects. It could reduce the chances of the emergence of advanced forms of diseases. Additionally, this combined therapy approach may offer enhanced protection against various environmental stressors and microbial threats, thereby bolstering overall health and well-being. Furthermore, exploring the

synergistic effects of these compounds could pave the way for novel therapeutic strategies with broader applications in both preventive and curative medicine.

REFERENCES

1. Donlan, R.M., Costerton, J.W. (2002): Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15: 167-193.
2. Hood, S.K., Zottola, E.A, (1995): Biofilms in food processing. Food Control 6: 9-18.
3. Frank, J.F. (2001): Microbial attachment to food and food contact surfaces. Adv. Food Nutr. Res. 43: 319-370.
4. Nikolić, M., Vasić, S., Đurđević, J., Stefanović, O., &Čomić, L. (2014). Antibacterial and anti-biofilm activity of ginger (Zingiber officinale (Roscoe)) ethanolic extract. *Kragujevac Journal of Science*, (36), 129-136.
5. Simões, M., Simões, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT-Food Science and Technology*, *43*(4), 573-583.
6. Quave, C.L., Plano L.R.W., Pantuso, T., Bennett, B.C. (2008): Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant Staphylococcus aureus. J. Ethnopharmacol. 118: 418-428.
7. Sandasi, M., Leonard, C.M., Viljoen, A.M. (2010): The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against Listeria monocytogenes. Lett. Appl. Microbiol. 50: 30-35.
8. Malsawmtluangi C, Nath DK, Jamatia I, Zarzoliana E, Pachuau L. (2013) Determination of Sun Protection Factor (SPF) number of some aqueous herbal extracts. J ApplPharmaceut Sci. 3(9), 15.
9. Bergman L, Nilsson B, Ragnarsson-Olding B, Seregard S. (2006) Uveal melanoma: a study on incidence of additional cancers in the Swedish population. Invest Ophth Vis Sci. 47(1), 72-77.

10. Shenoy PA, Khot SS, Chavan MC, Takawale JV, Singh S. (2010) Study of sunscreen activity of aqueous, methanol and acetone extracts of leaves of Pongamia pinnata (L.) Pierre, Fabaceae. Int J Green Pharm. 4(4).
11. Mishra AK, Mishra A, Chattopadhyay P. (2011). Herbal cosmeceuticals for photoprotection from ultraviolet B radiation: a review. Trop J Pharm Res. 10(3).
12. Lavania M, Dalal J, Cheema, S, Nautiyal CS, Lal B. (2011) In vitro study of lipid peroxidation and free radical scavenging activity of cow urine. Eur Food Res Technol. 232(4), 703-711.
13. Rekika D, Khanizadeh S, Deschênes M, Levasseur A, Charles MT, Tsao R, Yang R. (2005) Antioxidant capacity and phenolic content of selected strawberry genotypes. Hort Sci. 40(6), 1777-1781.
14. Ebrahimzadeh MA, Enayatifard R, Khalili M, Ghaffarloo M, Saeedi M, Charati JY. (2014) Correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents of some medicinal plants. Iran J Pharm Res. 13(3), 1041.
15. Kırmusaoğlu S. (2019) The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents.In *Exopolysaccharides-Methods of Preparation and Application*. IntechOpen.
16. Nikolić, M., Vasić, S., Đurđević, J., Stefanović, O., &Čomić, L. (2014). Antibacterial and anti-biofilm activity of ginger (Zingiber officinale (Roscoe)) ethanolic extract. *Kragujevac Journal of Science*, (36), 129-136.
17. Mansur MCPPR, Leitão SG, Lima LMTR, Ricci JE, Souza GR, Barbi NS, Martins TS, Dellamora GM, Leo RRT, Vieira RC, Leitão GG, Santos EP. (2012) Evaluation of the antioxidant and phototoxic potentials of Bauhiniamicrostachya var. massambabensis Vaz leaf extracts. Lat. Am J Pharm. 31, 200–206.
18. Sayre, R. M., Agin, P. P., LeVee, G. J., & Marlowe, E. (1979). A comparison of in vivo and in vitro testing of sunscreening formulas. *Photochemistry and Photobiology*, *29*(3), 559-566.
19. Adhikari N, Rana A, Oli S, Neupane S, Bhandari R, Joshi DR. (2020) Study of In-vitro Antioxidant and Antibacterial activity of leaf extract of Azadirachta indica, and Ocimum sanctum in different organic solvents and Cow urine. J Drug DelivTherapeut. 10(1), 90-95.

20. Ravikumar S, Nazar S, Nuralshiefa A, Abideen S. (2005) Antibacterial activity of traditional therapeutic coastal medicinal plants against some pathogens. J Environ Biol, 26, 383-386.
21. Deena MJ, Sreeranjini K, Thoppil JE. (2002) The in vitro microbial activity of the essential oils of Coleus aromaticus and Coleus zylanicus. Int J Aromatherapy. 12, 105-107.
22. Archana S, Abraham J. (2011) Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. JAppl Pharm Sci. 1(8), 149.
23. Ahuja A, Kumar P, Verma A and Tanwar RS. (2012) Antimicrobial activities of cow urine against various bacterial strains. Int J Recent Adv Pharm Res. 2(2), 84-87.
24. Nasceimento GGF, Locatelli J, Freitas PC and Silva GL. (2000) Antibacterial activity of plants extracts and photochemicals on antibiotic resistant bacteria. Braz J Microbiol. 31, 247-256.
25. Sirk TW, Brown EF, Friedman M, Sum AK. (2009) Molecular binding of catechins to biomembranes: relationship to biological activity. J Agric Food Chem. 57 (15), 6720–6728.
26. Cerca, N., Pier, G. B., Vilanova, M., Oliveira, R., &Azeredo, J. (2005). Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of Staphylococcus epidermidis. *Research in microbiology*, *156*(4), 506-514.
27. Pahal V, Kaur A, Dadhich KS. (2016) Synergistic antibacterial effect of some Indian medicinal plants and cow (Bos indicus) urine distillate against selective pathogenic gram-positive bacteria. Int J Pharm Sci Res. *7*(11), 4429.
28. Gupta, D. (2013). UV absorbing properties of some plant derived extracts. *Res. J. Chem. Environ. Sci*, *1*, 34-36.
29. Kaur CD, Saraf S. (2011) Photochemoprotective activity of alcoholic extract of Camellia sinensis. Int J Pharmacol. 7(3), 400-404.
30. Vinotha, M., Thavasuraj, S., & Nithya, V. (2021). Antimicrobial, antibiofilm and antioxidant effects of medicinal plants extract with indigenous cow ark against human pathogens. International Journal of Advanced Science and Technology; Vol. 29, No. 3, (2020), pp. 569 – 583.

31. Ebrahimzadeh, M. A., Nabavi, S.M., Nabavi, S.F., Bahramian, F. and Bekhradnia, A.,"Anti-oxidant and Free radical scavenging activity of H. officinalis L. var. angustifolius, V. odorata, B. hyracana and C. speciosum", Pakistan Journal of Pharmaceutical sciences,(2010), 23: 29-34.

32. Abalaka, M.E., Daniyan, S.Y., Oyeleke, S.B. and Adeyemo, S.O,"The antibacterial evaluation of Moringa oleifera Leaf extracts on selected bacterial pathogens", Journal of Microbiology Research,(2012),2:1-4.

33. Lavania, M., Dalal, J., Cheema, S., Nautiyal, C. S., & Lal, B. (2011). In vitro study of lipid peroxidation and free radical scavenging activity of cow urine. *European Food Research and Technology*, *232*, 703-711.

34. Athavale A, Jirankalgikar N, Nariya P, Des S. (2012) Evaluation of in-vitro antioxidant activity of panchagavya: a traditional ayurvedic preparation. Int J Pharm Sci Res. 3, 2543-9.

35. Ratnasooriya MD, Pieris KP, Samaratunga U and Jayakody JR (2004). Diuretic Activity of Spilanthesacmella flowers in Rats. J. Ethnopharmacol., 91: 317- 320.

36. Napagoda MT, Malkanthi BMAS, Abayawardana SAK, Qader MM, Jayasinghe L. (2016) Photoprotective potential in some medicinal plants used to treat skin diseases in Sri Lanka. BMC complementary alternat med. 16(1), 479.

37. Selka, M. A., Guendouz, S., Ouahrani, F., Mohammedi, A. Y., Yacineachouri, M., Chenafa, A., &Bellifa, N. SUNSCREEN FORMULATION BASED ON CAMELLIA SINENSIS LEAF EXTRACT. IJP, 2023; Vol. 10(5): 276-283.

38. Joshi DR, Aryal P, Chaudhary MK, Yadav SP, Rawal P, Bhandari R. (2019) Study of in-vitro Antioxidant and Antibacterial Activity of Cow Urine from Different Altitudinal and Climatic Region of Nepal. Microbiol Res J Int. 1-8.

39. Jarald E, Edwin S, Tiwari V, Garg R, Toppo E. (2008) Antioxidant and antimicrobial activities of cow urine. Global J Pharmacol. 2(2), 20-22.