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Phytochemical Analysis, Antioxidant and Antibacterial Activities of Some Medicinal Plants Growing in Chitrakoot

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Abstract

Plants used in traditional medicines are the best options for the discovery, development, and delivery of new drugs. Hence the present study was conducted to evaluate the antioxidant and antibacterial activities, chemical screening, and estimation of phenolics, flavonoids, tannins, and sugars in different extracts of *Ampelocissus daivaricata*, *Brassaiopsis hainla*, *Eranthemum pulchellum* and *Ficus cunia*. These plants are commonly used in Nepalese traditional medicine to treat various diseases.

Keywords- Traditional, Diseases, Drugs Etc.

Introduction

Chitrakoot is rich in various floras of medicinal value. Traditional medicine is still very popular in the rural areas of Chitrakoot, where only limited modern health facilities are available and people still rely on local medicinal plants to satisfy their primary healthcare needs. Overall, it is expected that about 75% of the rural population and about 75–80% of the world's population still rely on herbal medicines derived from medicinal plants for their primary health care [1,2]. The World Health Organization has been promoting traditional medicines as a source of less expensive, comprehensive medical care, especially in developing countries as they play an important role in the primary health care systems. Traditional medicine usually involves biological resources and the knowledge of the local healers regarding their medicinal use [3]. Traditional medicines are the best options for the discovery, development, and delivery of new drugs with enhanced performance, and still, many modern drugs are derived from natural products [4]. Natural products are not only considered direct sources of new pharmaceuticals but also provide unlimited opportunities for new drug leads because of their unmatched chemical diversity, structural complexity, and biological potency [5]. Plant-derived phytochemicals exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites. The production of these phytochemicals depends on the environmental conditions where they grow as well as genetics factors [6]. This chemical diversity, arising from the distribution of plant resources in different eco-geographic regions, has a direct impact on biological activities, which is very interesting to investigate [7]. In this study, we have selected four medicinal plants A. daivaricata, B. hainla, E. pulchellum, and F. cunia. A. daivaricata (Wall. ex M.A. Lawson) Planch, belongs to Vitaceae family. Traditionally, the root juice is used against snake or scorpion bites and root paste is applied externally on infected areas of skin [8,9]. B. hainla (Buch. -Ham. ex D. Don) Seem, which belongs to Araliaceae family. Roots are administered orally in dysentery [10]. E. pulchellum.

Andrews, belongs to Acanthaceae family. Extracts of leaf, stem, and root of this plant are utilized as antimicrobial and antiseptic agents [11]. *F. cinia* Buch. -Ham. ex Sm. (Synonym: *F. semicordata*) belongs to family Moraceae. Roots are also used to prevent menstrual disorders, leaf decoction is taken orally to get relief from jaundice, leaf juice is applied externally for curing scabies, treating stomach disorders, Anthelmintic, thrombolytic, cytotoxic, anti-diarrheal, and antipyretic properties of *B. hainla* have been reported. The antibacterial activity of silver nanoparticles of *E. pulchellum* has been reported and a new iridoid glucoside, *eranthemoside* has been isolated. Total phenolic, flavonoid, and tannin content as well as antioxidant, anticoagulant, antifungal, and hepatoprotective activities of *F. semicordata* have been reported. In this paper, we aim to bridge the traditional medicinal uses of these plants with the scientific findings. Therefore, we evaluate the total phenolic, flavonoid, tannin, sugar content, and antioxidant and antibacterial activities of *A. daivaricata*, *B. hainla*, *E. pulchellum* and *F. cunia*. The names of collected plants, collection sites, vernacular names, and voucher number are presented in Table 1. Collected plants, collection sites, vernacular names, and voucher number. Extraction The collected dried and powdered leaves of *A. daivaricata* (30 g), *B. hainla* (54 g), *E. pulchellum* (100 g), and *F. cunia* (78 g) were extracted successively with hexane (400 mL), dichloromethane (400 mL), ethyl acetate (300 mL) and methanol (300 mL) in a Soxhlet extractor for 6-7 hours until the last extract became colorless. The variable amount of dry leaves extracts used attributes to the number of samples collected. The remaining residue after extraction with methanol was refluxed with 50% aqueous methanol (200 mL) for 2 hours then allowed to cool and filtered. The respective extracts were concentrated under a rotary evaporator (BUCHI R-200, BUCHI V-800) at reduced pressure to obtain solid or semi-solid extracts. The dried extracts were kept in a fridge at -20 °C for further analysis.

Material and Method-

Sugar compounds (mg/g), c = concentration of gallic acid/($\pm$)- catechin/tannic acid/D-glucose established from the calibration curve in mg/mL, V = volume of extract (mL) and m = weight of plant extract. Determination of antioxidant activity The antioxidant activity of the extracts and ascorbic acid was determined using the DPPH free radical [30]. DPPH solution (0.10 mM), plant extracts and ascorbic acid solutions of different concentrations (5, 10, 20, 30, 40, 60, 80, and 100 μ g/mL) were prepared in methanol. To 0.5 mL of ascorbic acid or extract, 2.5 mL of DPPH solution was added with shaking and was kept in the dark for 30 min. Then absorbance was measured at 517 nm. A control was prepared by adding 0.5 mL methanol instead of ascorbic acid or extract. IC₅₀ value was calculated from the plotted graph of radical scavenging percentage against the concentration of ascorbic acid or extracts. Determination of antibacterial and antifungal activities - The antimicrobial activities of the extracts *aureus* (ATCC 25923), three Gram-negative bacteria, *S. typhi* (ATCC 14028), *E. coli* (ATCC25922), *S. sonnei* (ATCC25931) and a yeast species, *C. albicans* [31]. Mueller–Hinton agar was used for both bacteria and yeast. The extracts were prepared at a concentration of 100 mg/mL in 50% DMSO. Then 50 μ L of the prepared extract was introduced into the agar well of 6 mm diameter seeded with the respective microorganisms. Negative control experiments were performed using an equivalent volume of 50% DMSO and positive control experiments were performed using a standard antibiotic, ciprofloxacin for bacteria and amphotericin for yeast. At the end of the incubation period, the clear inhibition zone of bacterial growth was observed around each well in the presence of different extracts/standard drugs that were measured. Statistical analysis For total phenolic, flavonoid, tannin, sugar content, and antioxidant activity determination, absorbance data were recorded as a mean of three determinations for different concentrations.

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In the case where the results were statistically different ($p < 0.05$), Tukey-Kramer multiple comparison test was performed using Microsoft Excel 2016. Results and Discussion Extractive values in different solvents The extraction yield was found to be increased with solvent polarities. The highest amounts of extracts were obtained with methanol. This indicated that all the plants contain greater amounts of polar chemicals than the non-polar or less polar chemicals, *cunia* respectively. The results are presented in Table 2. Table 2. Percentage yields of various extracts. Phytochemical screening In phytochemical screening, most of the phytochemicals namely, polyphenols, flavonoids, tannins, terpenoids, alkaloids, saponins, quinones, glycosides, and reducing sugars were absent in hexane and dichloromethane extracts of all plants. Phenolics, flavonoids, and gallotannins were present in ethyl acetate, methanol, and 50% aqueous methanol extracts of all plants. Based on these phytochemical results, total phenolic, flavonoid, hydrolysable tannin and sugar content were estimated only for ethyl acetate, methanol and 50% aqueous methanol extracts of all plants. *A. daivaricata* to $(10.31 \pm 2.15 \text{ mg CE/g})$ for ethyl acetate extract of *E. pulchellum*. The variation in the content of phytochemicals depends on several factors. However, one of the important factors could be the standard compound used. Total hydrolysable tannin content The total tannin content in different extracts was calculated from the calibration curve using regression equation $y = 0.0037x + 0.0082$ and $R^2 = 0.9960$ and expressed as mg tannic Other extracts contained relatively lower amounts of tannins. The results are presented in Table 3. In the leaf extract of *F. semicordata*, total tannin content has been reported (587.00 ± 65.69) which was very high in comparison to our results [22]. In other plants, tannin content varies from $(123.1 \pm 0.39 \text{ mg TAE/g})$ in 50% aq. methanol extract of *A* n addition to other factors, generally, the content varies with the method applied for quantification. Gandhi et al. (2019) used Prussian blue colorimetric method to estimate the total tannin content, and we have used the FC method [22,39]. The results showed that higher amounts of sugars were present in the methanol $(324.56 \pm 0.299 \text{ mg GE/g extract})$ extract of *A. daivaricata*. Other extracts contained relatively lower amounts of sugars. The results are presented in Fig. 1. This indicated that the plant included in this study could be the source of bioactive polysaccharides. However, reports regarding sugar content in these plants were not available to date. Fig. 1. Total phenolics, flavonoids, tannins, and sugar content of plant extracts. DPPH antioxidant activityn addition, the methanol extract of *A. daivaricata* $(25.99 \pm 0.94 \text{ µg/mL})$, and ethyl acetate extract of *B. hainla* $(29.26 \pm 1.07 \text{ µg/mL})$ showed low IC50 values.

The results are presented in Table 3. Generally, the extracts having higher amounts of phenolics and flavonoids showed greater antioxidant Mean values $\pm$ SD (* $p < 0.05$ against positive control, ascorbic acid). Antimicrobial activity In antimicrobial assay, some of the extracts *A. daivaricata*, *B. hainla*, and *F. cunia* were active against gram-positive bacteria, *S. aureus* with an inhibition zone ranging between 7-12 mm. Only a few extracts were active against gram-negative bacteria with inhibition zones ranging between 8-12 mm. All extracts were found to be inactive against *E. coli*, only the dichloromethane extract of *B. hainla* was active against *S. typhi*. Dichloromethane extract of *A. daivaricata* and methanol extract of *E. pulchellum* were active against *K. pneumonia*. Only the extracts of *B. hainla* and *F. cunia* showed activity against *C. albicans* with an inhibition zone ranging between 7-10 mm.

The results are presented in Table 4. Gandhi et al. (2019) also reported the antifungal activity of methanol extract of the leaf of *F. semicordata* against *C. albicans* with an inhibition zone of 11 mm while our extract showed an inhibition zone of 8 mm. The greater activity of *F. semicordata* against *C. albicans* could be due to the presence Zone of inhibition produced in mm including 6 mm well. Plant extracts are a complex mixture of various phytochemicals, they show synergistic as well as antagonistic effects [41, 42]. The weak activity of our extracts could be due to the antagonistic effect where some phytochemicals reduce the effect of others. So, it is necessary to isolate and characterize the bioactive compounds. The findings of this study support to some extent, the traditional use of these plants to treat stomach disorders due to bacterial infections, skin rashes, and scabies due to fungal infections.

Conclusion-

plants *A. daivaricata*, *B. hainla*, *E. pulchellum*, and *F. cunia*. The finding of this study revealed that all plants are the sources of phenolics, flavonoids, tannins, and sugars. However, the extracts of *A. daivaricata* and *F. cunia* are rich *F. cunia*, methanol extract of *A. daivaricata*, and ethyl acetate extract of *B. hainla* showed low IC₅₀ values. These extracts contain higher amounts of phenolics and flavonoids. Thus, these plants could be the source of antioxidants to manage oxidative stress-induced health disorders. However, in statistical analysis, using one-way ANOVA followed by Tukey test, there were significant differences in IC₅₀ values between the different extracts and standard ascorbic acid. In an antimicrobial assay, all the extracts showed a very weak and narrow spectrum of activity. Some extracts of *A. daivaricata*, *B. hainla*, and *F. cunia* were active against gram-positive bacteria. Only a few extracts of *A. daivaricata*, *B. hainla*, and *E. pulchellum* were active against gram-negative bacteria. Only ethyl acetate and methanol extracts of *B. hainla* and *F. cunia* showed activity against *C. albicans*.

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