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Research Article

Pharmacological activities of six species of *Hedychium* J. Koenig from Nepal

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Abstract

Species of *Hedychium* Koenig are perennial herbs. Some of the species like *H. ellipticum*, *H. spicatum*, *H. coronarium* are traditionally used as medicinal plants. In the present study, methanolic rhizome extracts of six different species of *Hedychium* namely *H. spicatum*, *H. ellipticum*, *H. thyrsiforme*, *H. coccineum*, *H. gardnerianum* and *H. coronarium* were analysed for their antioxidant, antidiabetic and antibacterial activities. The antioxidant activity analysed by DPPH assay showed the highest potential (lowest IC_{50} value) in *H. coccineum* (148.82±2.83 µg/ml) and lowest potential (maximum IC_{50} value) in *H. thyrsiforme* (996.55±9.42 µg/ml). The rhizome extracts of different species showed moderate α-amylase inhibition activity *in vitro*. The highest α-amylase inhibition (79.67%) was observed for *H. coronarium* while the lowest inhibition (64.0%) was observed in *H. thyrsiforme.* However, these values were found lower than the value (92.37%) obtained for positive control, i.e., Acarbose. The antibacterial activity was determined against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacterial strains by agar well diffusion method. Except for *H. ellipticum* the extracts of all other species showed antibacterial activity against all the bacterial strains tested. The extracts of *H. ellipticum* showed antibacterial activity only against *B. subtilis* and *K. pneumoniae*. The extract of *H. coronarium* showed the highest zone of inhibition (16.67±1.15 mm) against *B. subtilis*. However, the antibacterial activity was weak compared to standard antibiotics for all the extracts and at all concentration tested. These results show that rhizomes of other species can also be used in the same manner as that of *H. coronarium* and *H. spicatum*, two species most used in various ethnomedicinal applications.

Keywords: Biological activity, Rhizome extract, Hedychium, Himalaya

Introduction

The species of *Hedychium* J. Koenig belongs to the family Zingiberaceae (Ginger family). These plants are native to Asia and distributed throughout tropical Africa and America (Chang, 2017). Out of around 65 species of *Hedychium* worldwide, 10 species are reported from Nepal (Rajbhandari & Rai, 2017). These species are distributed from tropical (150 m) to sub-alpine (3500 m) regions. Among the species reported from Nepal, five species namely *H. coccineum*, *H. coronarium*, *H. ellipticum*, *H. gardnerianum* and *H. spicatum* are reported to have been used in traditional medicine.

The rhizome of *Hedychium* has ethnomedicinal applications. Juice from the rhizomes of species like *H. ellipticum, H. gardnerianum* and *H. spicatum* is used in fever in Nepal (Manandhar, 2002; DPR, 2016). Decoction of rhizomes of *H. spicatum* is used in cough and cold by the Newar communities in Pharping, Nepal (Balami, 2004). Similarly, rhizomes of *H. spicatum* are used in stomachache, indigestion, loss of appetite, constipation, etc. by Raji communities in far western Nepal (Thapa et al., 2014). Additionally, it is also used in the treatment of dyspepsia, nausea and pain, tuberculosis, asthma, foul breath, bronchitis, hiccoughs, blood disease, and poor circulation (Rawat et al., 2018). Likewise, the rhizome of *H. coronarium* is also very popular in traditional medicine practice and used in diabetes and diphtheria (Bhandary et al., 1995), in headaches (Pattanaik et al., 2008), and in vomiting (Devi et al., 2014). The rhizomes of *H. coccineum* are used as medicine for swelling caused by bruises and wounds (Tushar et al., 2010, Basak et al., 2010). The rhizomes of *H. spicatum*, have been used in liver complaints, diarrhoea, inflammation, pain, snake bites, etc. (Tushar et al., 2010).

Rhizome extracts or essential oils from *H. coronarium, H. gardnerianum* and *S. spicatum* have been reported to show antioxidant, antidiabetic and antibacterial activity in various studies. Rhizome extracts and essential oils of *H. coronarium* and *H. gardnerianum* have antioxidant and antibacterial (Zhao et al., 2017; Ray et al., 2018). Antioxidant activity has also been reported in rhizome extracts of *H. spicatum* (Bag et al., 2015). Essential oil of *H. spicatum* (Reddy et al., 2009; Kaur & Richa, 2017) and rhizome extract of *H. coronarium* (Panigrahy et al., 2020) have also been reported to show antidiabetic activity. Similarly, the rhizome extract of *H. spicatum* (Lamichhane et al., 2014; Arora & Mazumder, 2017) and *H. coronarium* (Ho, 2011; Sah et al., 2012) has antibacterial activity. Antibacterial activity has also been reported in the essential oil of *H. spicatum* (Prakash et al., 2010), *H. coronarium* (Ray et al., 2018) and *H. gardnerianum* (Prakash et al., 2010).

Earlier studies have been confined mainly to selected medicinally known species such as *H. spicatum* and *H. coronarium* and focused more on the activities of their essential oils. Therefore, the present study is aimed to screen methanolic rhizome extracts of different species of *Hedychium* from Nepal for antioxidant, antidiabetic and antibacterial activities, and to compare such activities between ethnomedicinally important species and others.

Materials and Methods

Plant materials

Plant samples of five species were collected from Kathmandu and Parbat. Voucher specimens were deposited at TUCH for future reference. The details about the collection of *Hedychium* species are mentioned in Table 1.

Preparation of plant extracts and extract dilution

Rhizomes of *Hedychium* species (Figure 1) were cleaned, peeled, chopped and shade-dried separately. Rhizome powder (4 gm) of each plant sample was taken separately in Falcone tubes and 40 ml of methanol was poured into it. The mixture was sonicated in an Ultrasonicator (E-Chrom Tech, Taiwan UC-7240BDT) for 2 hours at 40°C. Each mixture was filtered by using filter paper (Whatman No. 1). The filtrate was collected, and the residue was sonicated again for 1 and ½ hours. The mixture was filtered and collected filtrates were mixed and then concentrated under reduced pressure by using a rota-evaporator (RE 100 PRO, DRAGON Lab, China). The concentrated filtrate was poured into a pre-weighed petriplate and left for drying under aseptic conditions. The dried extract was weighed and kept in 2 ml polypropylene tubes at -20°C.

Table 1: Different species of *Hedychium* used in the study and their locality.

S.N.	Plant Name	Locality	Altitude (m)	GPS coordinates
1.	*H. coccineum* Buch.-Ham. ex Sm.	Chalnakhel, Kathmandu	1415	27°37'57"N, 85°16'49"E
2.	*H. coronarium* J. Koenig	Kirtipur, Kathmandu	1311	27°40'56"N, 85°16'38"E
3.	*H. ellipticum* Buch.-Ham. ex Sm.	Chalnakhel, Kathmandu	1415	27°37'57"N, 85°16'49"E
4.	*H. gardnerianum* Shepperd ex Ker Gawl.	Chalnakhel, Kathmandu	1355	27°38'00"N, 85°16'45"E
5.	*H. spicatum* Sm.	Kyang, Parbat	1768	28°17'59" N, 83°41'8"E
6.	*H. thyrsiforme* Buch.-Ham. ex Sm.	Chalnakhel, Kathmandu	1415	27°37'57"N, 85°16'49"E

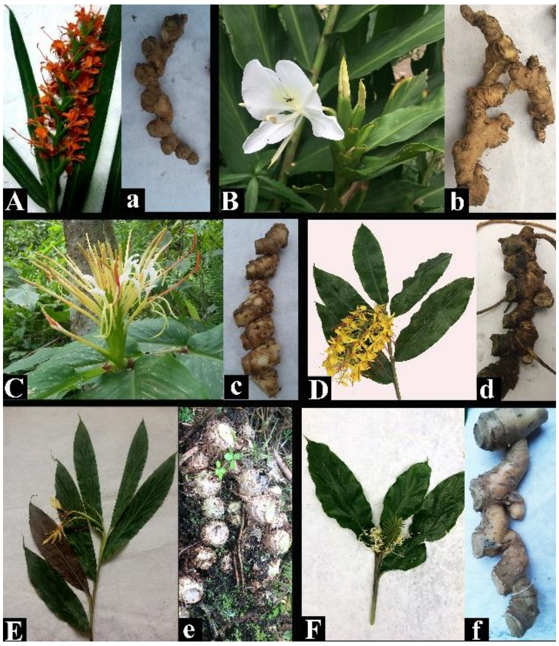

Figure 1: Plant specimen and rhizomes of selected *Hedychium* species: *H. coccineum* (A, a), *H. coronarium* (B, b), *H. ellipticum* (C, c), *H. gardnerianum* (D, d), *H. spicatum* (E, e), H. *thyrsiforme* (F, f). Uppercase: aerial part, and Lowercase: rhizome.

Antioxidant activity

For evaluating the antioxidant activity, the radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used following Blois (1958) with modification. A fresh solution of 0.2 mM DPPH in methanol was prepared. Different concentrations of ascorbic acid (10-100 µg/ml) or rhizome extract (25-200 µg/ml) were prepared in methanol. Then, 500 µl of sample (ascorbic acid or plant extract) was mixed with 500 µl of DPPH solution. The mixture was shaken well and placed in the dark for 30 minutes at room temperature. Then absorbance was measured at 517 nm. The blank was prepared by replacing plant extract or ascorbic acid with methanol. The percentage of free radical scavenging activity (RSA) of the plant samples was calculated by using the following formula:

% RSA = (control absorbance − sample absorbance) / control absorbance × 100

A curve was obtained by plotting the percentage RSA against concentration. Based on the standard curve, IC_{50} was calculated by using a linear equation of the curve obtained.

$IC_{50} = (0.5 - b)/a$

Where, X = Concentration, Y = % RSA, a and b are the coefficient and constant, respectively of the linear equation

α-amylase inhibition assay

The antidiabetic activity was measured by using a standard α-amylase assay based on increasing the reducing power of starch under the influence of the enzyme (Bernfeld, 1955) with modification. The reaction medium was prepared by dissolving porcine pancreatic amylase (Sigma Aldrich, Germany) in 0.1M potassium phosphate buffer (pH 6.8) to a final concentration of 0.1 unit/ml. Then 390 µl of reaction medium was added to 10 µl of either pure solvent (control/no inhibition) or acarbose (ARISTO Pharmaceutical Pvt. Ltd., India) or plant extracts solution in methanol (1 mg/ml) in two separate test tubes. In one of the test tubes (blank) 200 µl of DNS reagent (Sigma Aldrich, Germany) was added to the above reaction mixture. The tubes were pre-incubated at 37°C for 10 minutes. Then 200 µl of 1% soluble starch (Fisher Scientific, India) was added in all the tubes and incubated at 37°C for another 20 minutes. Then, 200 µl of DNS reagent was added to all the remaining tubes. The tubes were kept in a boiling water bath for 10 minutes and then allowed to cool. Then, 4 ml of distilled water was added to each tube and absorbance was taken at 540 nm in a UV/Vis spectrophotometer (E-Chrom Tech, Taiwan) using respective blanks. Inhibition of amylase activity was calculated by using the following formula.

% inhibition of amylase activity = (control absorbance - sample absorbance) / (control absorbance) × 100

Antibacterial activity

Bacterial strains: Various bacterial strains were obtained from Madhyapur Hospital, Thimi, Bhaktapur and the National Public Health Laboratory (GoN), Teku Kathmandu.

Gram-negative bacterial strains: *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603)

Gram-positive bacterial strains: *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (clinical sample)

Agar well diffusion method: The antibacterial test was performed by the modified agar well diffusion method (Perez et al., 1990). The rhizome extracts of each species were dissolved in DMSO to make a stock of 100 mg/ml. The stock was diluted to 50 mg/ml, 25 mg/ml and 12.5 mg/ml by serial dilution. The name of the bacterial strain, the name and concentration of plant extracts and the date were labelled on Muller Hinton Agar (MHA) plates (HiMedia Laboratories, Mumbai India). Then, six wells were prepared by using a cork borer in each petriplate; four wells for different concentrations of plant extracts, 1 each for DMSO (negative control) and 10 µg gentamicin (HiMedia Laboratory Pvt. Ltd., India) as a positive control. The sterilized filter paper discs and 1 gentamicin disc were placed in wells by using sterilized forceps. Then 30 µl extract of each concentration was poured into wells. The cotton swab was dipped in the fresh suspension culture in Nutrient Broth (HiMedia Laboratories, Mumbai India) adjusted to 0.5 Mcfarland standard, and swabbed on the labelled MHA petriplates under aseptic conditions. The petriplates were allowed to dry and closed tightly by using Parafilm (Bemis, USA). The plates were incubated on the microbial incubator overnight at 37°C and the zone of inhibition was measured by using a scale.

Data analysis

All data were taken in triplicate and statistically analyzed using Microsoft Excel.

Results and Discussion

Antioxidant activity

The antioxidant activity of ascorbic acid and plant extracts of different concentrations is presented in Figure 2 and Figure 3 respectively. Among the plant extracts, free radical scavenging activity was reported to range from 8.92±0.48% (*H. thyrsiforme*) to 62.32±3.05% (*H. coccineum*) at 200 µg/ml (Fig. 3). The free radical scavenging activity was increased with increasing concentration of the crude extracts.

The IC_{50} value of selected species of *Hedychium* is shown in Figure 4. The IC_{50} value was reported to range from 148.82± 2.83 µg/ml (*H. coccineum*) to 996.55±9.42 µg/ml (*H. thyrsiforme*). The extract with the lowest IC_{50} value for *H. connineum* was found to show the best antioxidant activity.

The antioxidants can reduce oxidative stress and help in the prevention and treatment of related diseases (Sun et al., 2018). The phenolic compounds (flavonoids, phenolic acids, stilbenes, tocopherols, tocotrienols), ascorbic acid, carotenoids and terpenoids are naturally occurring antioxidants in plants (Grassmann, 2005; Dubey et al., 2015). The free radical scavenging activity of *H. spicatum* was comparable to the study of Lock et al. (2005) at 50 µg/ml. A lower IC_{50} value was reported in the present study for methanolic extract of *H. spicatum* rhizomes than in the study of Sravani & Paarakh (2012). The higher free radical scavenging activity of *H. spicatum* than *H. coronarium* in the present study supports the findings of Bag et al. (2015). Variation in radical scavenging activity in the present study was not comparable with previous findings due to the different concentrations of samples and solvents used (Ho, 2011; Zhao et al., 2017). The variation in IC_{50} value among different species of *Hedychium* may be due to variations in phenolic compounds (Grassmann, 2005; Dubey et al., 2015; Bag et al., 2015).

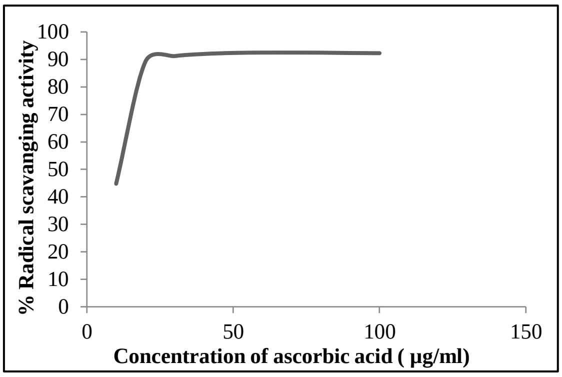

Figure 2: Standard curve of ascorbic acid.

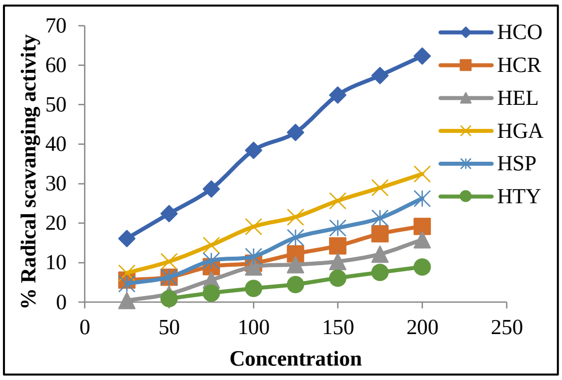

Figure 3: Percentage of free radical scavenging activity of extracts of *Hedychium* species. **Legend:** HCO- *H. coccineum*, HCR- *H. coronarium*, HEL- *H. ellipticum*, HGA- *H. gardnerianum*, HSP- *H. spicatum*, HTY- *H. thyrsiforme*.

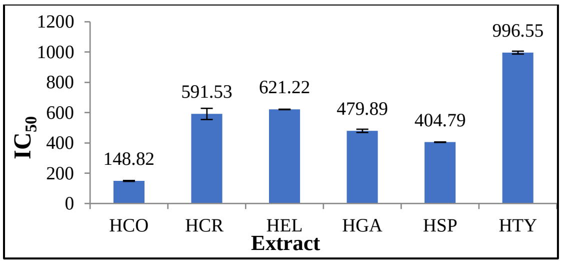

Figure 4: IC_{50} values of extracts of *Hedychium* species. **Legend:** HCO- *H. coccineum*, HCR- *H. coronarium*, HEL- *H. ellipticum*, HGA- *H. gardnerianum*, HSP- *H. spicatum*, HTY- *H. thyrsiforme*.

α-amylase inhibition activity

In general, all the tested species of *Hedychium* possessed moderate α-amylase inhibition activity (Figure 5). *H. coronarium* extract showed the highest (79.67%) while *H. thyrsiforme* extracts showed the lowest (64.0%) inhibition of α-amylase. Acarbose showed 96.19±1.28% at the same concentration. The percentage inhibition of α-amylase in extracts of all selected species was lower than that of acarbose, the standard antidiabetic drug.

Although the antidiabetic activity of *H. spicatum* and *H. coronarium* was studied earlier, the present study is not comparable due to differences in assay protocols and the use of bioactive compounds instead of crude extract (Reddy et al., 2009; Panighrhy et al., 2020). In the present study, moderate inhibition of in vitro α-amylase activity suggests that inhibition of enzymes of carbohydrate metabolism is possibly one of the mechanisms through which these plants show antidiabetic activities in vivo. This study supports the traditional use of *H. coronarium* rhizomes in diabetes. The result of the present study suggests that the rhizomes of other species can also be used in the same manner as that of *H. coronarium* for antidiabetic activity.

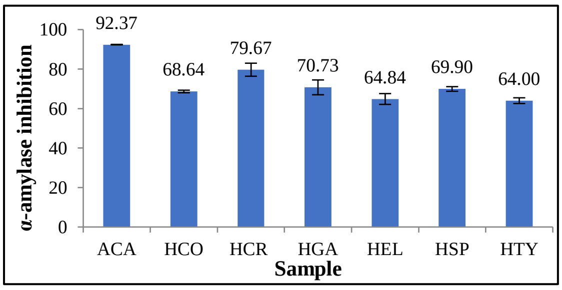

Figure 5: α-amylase inhibition activity of acarbose and extracts of *Hedychium* species (10 µg each). **Legend:** ACA- Acarbose, HCO- *H. coccineum*, HCR- *H. coronarium*, HEL- *H. ellipticum*, HGA- *H. gardnerianum*, HSP- *H. spicatum*, HTY- *H. thyrsiforme*.

Antibacterial activity

The antibacterial activity of methanolic extracts of rhizome of selected species of *Hedychium* against Gram-negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains are shown in Table 2. The extract of *H. coronarium* at 100 mg/ml showed the highest zone of inhibition against *S. aureus*, *B. subtilis* and *P. aeruginosa* i.e., 14.7±1.15 mm, 16.7±1.15 mm and 17.6±0.58 mm, respectively. Extracts of *H. ellipticum* did not even show inhibition of bacterial growth except in *B. subtilis*. Extracts of other species showed less inhibition zone against tested bacteria. In comparison to the positive control (10 µg of gentamicin), all extracts were found less effective. The antibacterial activity of extracts of tested species of *Hedychium* was much weaker compared to positive control even at very high concentrations of the extract.

The plants synthesize several classes of secondary metabolites, to defend against pathogens like fungus, bacteria, viruses and nematodes (Taiz & Zeiger, 2010). Some of these can be used as potent antibiotics to treat various infections. The agar well diffusion test method is one of the most practical methods routinely used to find out the antimicrobial potential of plant extracts. The same method has also been used in determining the antimicrobial potential of various species of *Hedychium*. However, the antimicrobial activities of rhizome extracts of *Hedychium* species have been carried out in different solvents like methanol, ethyl acetate, petroleum ether, dichloromethane, ethanol and water (Aziz et al., 2009; Bisht et al., 2006; Ho, 2011; Sah et al., 2012; Lamichhane et al., 2014) with methanol being the solvent of choice in most of the studies possibly due to its low cost and ability to dissolve most of the secondary metabolites. The zone of inhibition of different bacterial strains in rhizome extracts of *Hedychium* species have been reported in range of 11 to 18 mm for *S. aureus*, 15 to 18 mm for *B. subtilis* (Sah et al., 2012; Aziz et al., 2009; Ho, 2011), 10 to 19 mm for *P. aeruginosa* (Chen et al., 2008; Aziz et al., 2009; Ho, 2011) and 13 mm for *Klebsiella pneumoniae* (Sah et al., 2012). The findings of the present study are comparable to those of Aziz et al. (2009), Ho (2011) and Sah et al. (2012). Arora & Mazumder (2017), however, have reported higher antibacterial activity of *H. spicatum* extract. Since the secondary metabolites content in plant extracts is affected by several factors such as genotype, physiological status of the plant, harvesting period, extraction methods, any of these factors might have been the reason behind such discrepancies in the antibacterial activities of extracts of even the same species.

Table 2: Antibacterial activity of *Hedychium* extracts in different bacterial strains.

Rhizome extract	Bacterial strain	Zone of inhibition in mm including diameter of a well (5 mm)				
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Gentamicin (10 µg)
H. coccineum	*K. pneumoniae*	9.3±0.58	7.7±1.15	5	5	23±0
	P. aeruginosa	9.7±1.15	8.0±0	7.0±0	6±0	22±0
	B. subtilis	10.7±0.58	9.7±0.58	7.0±0	7.0±0	23.0±0
	S. aureus	12±1.73	9.3±0.58	8.0±0	7.0±0	15.0±0
H. coronarium	*K. pneumoniae*	15±1	13±1	12±1.73	10.7±2.08	23±0
	P. aeruginosa	17.6±0.58	16.7±0.58	15.7±0.58	14.7±0.58	22±0
	B. subtilis	16.7±1.15	15.7±1.15	14.3±0.58	13.00	22.3±1.15
	S. aureus	14.7±1.15	13.0±0	11.0±0	10.0±0	15.7±1.15
H. ellipticum	*K. pneumoniae*	7±0	5	5	5	22.7±0.58
	P. aeruginosa	5	5	5	5	21±0
	B. subtilis	9.3±0.58	8.3±0.58	7.3±0.58	7±1	22±0
	S. aureus	5	5	5	5	15±0
H. gardnerianum	*K. pneumoniae*	11±1	9±0	8±0	7±0	23±0
	P. aeruginosa	10.3±0.58	9.3±0.58	8.3±0.58	7.3±0.58	22±0
	B. subtilis	10.7±1.15	9.7±1.15	8.7±1.15	7.7±1.15	22.3±0.58
	S. aureus	8.7±0.58	7.7±0.58	7.0±0	5	15.0±0
H. spicatum	*K. pneumoniae*	15.3±0.58	13±0.57	10±0	9±0.58	22.7±0.58
	P. aeruginosa	12±0	10.3±0.68	9.33±0.58	7±0	21.3±0.58
	B. subtilis	11.7± 0.57	10.7±0.58	9.0±1	5	22±1
	S. aureus	10±0	9±0	5	5	15±0
H. thyrsiforme	*K. pneumoniae*	11±0	9±0	8±0	6±0	23±0
	P. aeruginosa	11.3±0.58	9±0	8±0	5	21.7±0.58
	B. subtilis	11.0±0	10.0±0	9.0±0	8.0±0	19.5±0.58
	S. aureus	10.3±0.58	9.0±0	8.0±0	5	15.0±0

Conclusion

The present investigation of rhizome extracts of six species of *Hedychium* reveals weak antioxidant and antibacterial activity, and moderate α-amylase inhibition activity. Among the species, *H. coccineum* was the best in terms of antioxidant activity while *H. coronarium* was best in terms of antidiabetic (α-amylase inhibition) and antibacterial activity. Since the species showed antioxidant, antibacterial and antidiabetic activities, there is a possibility of using them as substitutes in traditional medicine.

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References

DPR. (2016). *Medicinal plants of Nepal*. Ministry of forest and soil conservation, Department of Plant Resources, Thapathali, Kathmandu, Nepal.

Arora, R., & Mazumder, A. (2017). Phytochemical screening and antimicrobial activity of rhizomes of *Hedychium spicatum*. *Pharmacognosy Journal*, *9*(6s), s64-s68.

Aziz, M. A., Habib, M. R., & Karim, M. R. (2009). Antibacterial and cytotoxic activities of *Hedychium coronarium* J. Koenig. *Research Journal of Agriculture and Biological Sciences*, *5*(6), 969-972.

Bag, G. C., Devi, P. G., & Bhaigyabati, T. H. (2015). Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three *Hedychium* species of Manipur valley. *International Journal of Pharmaceutical Sciences Review and Research*, *30*(1), 154-159.

Balami, N. P. (2004). Ethnomedicinal uses of plants among the Newar community of Pharping village of Kathmandu District, Nepal. *Tribhuvan University Journal*, *24*(1), 13-19.

Bernfeld, P. (1955). Amylases, α and β. In S. P. Colowik, & N. O. Kaplan (Eds.), *Methods in Enzymology*: *Volume 1* (pp. 149-225). Academic Press.

Bhandary, M. J., Chandrashekar, K. R., & Kaveriappa, K. M. (1995). Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India. *Journal of Ethnopharmacology*, *47*(3), 149-158.

Bisht, G. S., Awasthi, A. K., & Dhole, T. N. (2006). Antimicrobial activity of *Hedychium spicatum*. *Fitoterapia*, *77*(3), 240-242.

Blois, M. S. (1958). Antioxidant determination by the use of stable freeradicals. *Nature, 181*, 1199-1200.

Chang, L. (2017). Zingiberaceae. In Z. Xu & L. Chang, (Eds.), *Identification and Control of Common Weeds: Volume* 3 (pp. 909-911). Springer, Singapore.

Chen, I. N., Chang, C. C., Ng, C. C., Wang, C. Y., Shyu, Y. T., & Chang, T. L. (2008). Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods for Human Nutrition*, *63*(1), 15-20.

Devi, N. B., Singh, P. K., & Das, A. K. (2014). Ethnomedicinal utilization of Zingiberaceae in the valley districts of Manipur. *IOSR Journal of Environmental Science Toxicology and Food Technol*ogy, *8*(2), 21-3.

Dubey, N. K., Kedia, A., Prakash B., & Kishore, N. (2015). Plants of Indian traditional medicine with antioxidant activity. In N. K. Dubey (Ed.), *Plants as a Source of Natural Antioxidants* (pp 1-14). CABI International.

Grassmann, J. (2005). Terpenoids as plant antioxidants. *Vitamins & Hormones*. *72*, 505-535.

Ho, J. C. (2011). Antimicrobial, mosquito larvicidal and antioxidant properties of the leaf and rhizome of *Hedychium coronarium*. *Journal of the Chinese Chemical Society*, *58*(4), 563-567.

Kaur, H., & Richa, R. (2017). Antidiabetic Activity of Essential Oil of *Hedychium spicatum*. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(6), 853-857.

Lamichhane, J., Chhetri, S. B., Bhandari, M., Pokhrel, S., Pokharel, A., & Sohng, J. K. (2014). Ethnopharmacological survey, phytochemical screening and antibacterial activity measurements of high-altitude medicinal plants of Nepal: A bioprospecting approach. *Indian Journal of Traditional Knowledge. 13*(3): 496-507.

Lock, O., Castillo, P., Doroteo, V., & Rojas, R. (2005). Antioxidant activity in vitro of selected Peruvian medicinal plants. *Acta Horticulturae, 675*, 103-106.

Manandhar, N. P. (2002). *Plants and People of Nepal*. Timber Press Inc, USA

Panigrahy, S. K., Kumar, A., & Bhatt, R. (2020). In vitro and In vivo anti-diabetic Activity of Fractions obtained from the unexplored Hedychium coronarium Rhizome. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *90*, 605-614.

Pattanaik, C., Reddy, C. S., & Murthy, M. S. R. (2008). An ethnobotanical survey of medicinal plants used by the Didayi tribe of Malkangiri district of Orissa, India. *Fitoterapia*, *79*(1), 67-71.

Perez, C., Pauli, M., & Bazerque, P. (1990). An antibacterial assay by agar well diffusion method. *Acta Biologiae et Medicinae Experimentalis*, *15,* 113-115.

Prakash, O., Rajput, M., Kumar, M., & Pant, A. K. (2010). Chemical composition and antibacterial activity of rhizome oils from *Hedychium coronarium* Koenig and *Hedychium spicatum* Buch-Ham. *Journal of Essential Oil Bearing Plants*, *13*(2), 250-259.

Rajbhandari, K. R., & Rai, S. K. (2017). *Handbook of the flowering plants of Nepal*. Volume 1. Department of Plant Resources. Government of Nepal. Thapathali, Kathmandu.

Rawat, S., Jugran, A. K., Bhatt, I. D., & Rawal, R. S. (2018). *Hedychium spicatum*: A systematic review on traditional uses, phytochemistry, pharmacology and future prospects. *Journal of Pharmacy and Pharmacology*, *70*(6), 687-712.

Ray, A., Jena, S., Dash, B., Kar, B., Halder, T., Chatterjee, T., Ghosh, B., Panda, P. C., Nayak, S., & Mahapatra, N. (2018). Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of Hedychium coronarium Koen. *Industrial Crops and Products*, *112*, 353-362.

Reddy, P. P., Tiwari, A. K., Rao, R. R. Madhusudhana, K., Subba Rao, V. R., Ali, A. Z., Babu, K. S., & Rao J. M. (2009). New Labdane diterpenes as intestinal α-glucosidase inhibitor from antihyperglycemic extract of *Hedychium spicatum* (Ham. Ex Smith) rhizomes. *Bioorganic & Medicinal Chemistry Letters*, *19*(9), 2562-2565.

Sah, S., Shrestha, R., Koirala, S., & Bhattarai, K. (2012). Phytochemical and antimicrobial assessment of five medicinal plants found in Terai Region. *Nepal Journal of Science and Technology*, *13*(2), 79-86.

Sravani, T., & Paarakh, P. M. (2012). Antioxidant activity of *Hedychium spicatum* Buch.-Ham. rhizomes. *Indian Journal of Natural Products and Resources*, *3*(3), 354-358.

Taiz, L., & Zeiger, E. (2010). Plant physiology 5th Ed. Sinauer Associates Sunderland, MA.

Thapa L. B., Dhakal, T. M., Chaudhary, R., & Thapa, H. (2014). Medicinal plants used by Raji ethnic tribe of Nepal in treatment of gastrointestinal disorders. *Our Nature*. *11*(2), 177-186.

Tushar, Basak, S., Sarma, G. C., & Rangan, L. (2010). Ethnomedical uses of Zingiberaceous plants of Northeast India. *Journal of Ethnopharmacology*, *132*(1), 286-296.

Zhao, M., Zhang, L., Yang, Z., Wei, J., Zhang, K., Zheng, X., Fang, Y., Lin, L., Tang, J., Wu F., & Du, Z. (2017). Antioxidative activities of essential oils and ethanol extractions from ornamental Zingiberaceae species. *Journal of Essential Oil Bearing Plants*, *20*(1), 215-222.