ISSN : 1001-1781
Journal of Clinical Otorhinolaryngology, Head, and Neck Surgery

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *RHYNCHOSIA HEYNEI WIGHT & ARN* WHOLE PLANT EXTRACTS: *IN VITRO*

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Abstract

Bacterial and fungal infections are a significant challenge for public health across the world, andrise of antibiotic-resistant strains has made it necessary to search for new sources of antibiotics and antifungal agents. Therefore, this study intends to explore. the antibacterial and antifungal activities of R. heynei whole plant extracts using *in vitro* assays. The ethanol extract of R. heynei was found to exhibit the maximum amount of antimicrobial activity against S. aureus, with zone of inhibition diameter of 15 ± 1.41 mm at 500 µg/disk concentration. Similarly, the diethyl ether extract showed potent antibacterial activity against S. aureus, with an inhibition zone diameter of 13.5 ± 0.70 mm at 1000 µg/disk concentration. The hexane extract was found to be most effective against P. aeruginosa, with an inhibition zone diameter diameter of 11 ± 0.00 mm at 1000 µg/disk concentration. Furthermore, the antifungal activity of R. heynei extracts against C. albicans and A. niger was also assessed, and the results showed that the plant extracts possessed decent antifungal properties. The R. heynei extracts showed a wide range of antibacterial activity, with differing degrees of efficacy against various microorganisms. Significantly, extreme susceptibility to the Gram-positive bacteria S. aureus and the Gram-negative bacteria E. coli was discovered in the examined extractssuggest the potential for R. heynei extracts to be turned into efficient antibacterial agents; however, further research is required.

Keywords: Rhynchosiaheynei Wight & Arn, Antibacterial, antifungal activity, Zone inhibition, Plant extracts.

Introduction

Infections caused by microorganisms and fungi are a leading cause of death and disability globally. Antibiotic-resistant microbial strains have emerged because of the overuse of these drugs, which is a growing public health concern. In this context, natural drugs have surfaced as an attractive option to synthetic drugs for the treatment of microbial and fungal infections. Natural drugs are compounds derived from natural sources such as plants, animals, and microorganisms that possess antimicrobial properties. Natural drugs possess a wide range of antimicrobial properties that make them effective against a broad spectrum of

microorganisms. Plants are the most common source of natural drugs, and many plant extracts have been shown to include properties that make them effective in killing microbes [1]. Additionally, some animal and microbial-derived natural drugs have also been found to have potent antimicrobial activity. For example, amphibian skin secretions have been found to contain a variety of peptides with antibacterial effect on a wide variety of bacteria and viruses [2]. Moreover, some fungal metabolites, such as penicillin and cephalosporins, have been used as antibiotics for decades. Natural drugs have several advantages over synthetic drugs, making them an attractive option for the treatment of microbial and fungal infections. One advantage is that natural drugs are generally considered safe and have fewer side effects compared to synthetic drugs [3]. Additionally, natural drugs are often more readily available and less expensive than synthetic drugs. Furthermore, natural drugs have a lower risk of developing antibiotic resistance because they often contain complex mixtures of compounds that act synergistically to kill microorganisms [4].The search for new antimicrobial agents has become increasingly urgent as antibiotic resistance continues to grow. Natural drugs are a promising source of new antimicrobial agents. Many natural drugs have not been extensively studied, and there is a wealth of untapped potential in natural sources. Additionally, the complex mixtures of compounds found in natural drugs often provide a greater diversity of chemical structures, which can be used to develop new drugs with unique properties [5].

Rhynchosiaheynei is a plant species family Fabaceae, which includes a vast variety of plants found throughout India, Sri Lanka, and other parts of South Asia. This plant has been traditionally used for tohe treatment of various ailments, including fever, wounds, and skin diseases [6]. Phytochemical analysis of R. heynei revealed the presence of various classes of compounds including flavonoids, terpenoids, steroids, alkaloids, and tannins [7]. The plant has been reported to display multiple pharmacological effects, such as anti-inflammatory, anti-oxidant, and other effects, wound healing, anticancer, antidiabetic, and hepatoprotective effects [8-14]. Several studies have investigated the bioactive compounds and pharmacological potential of R. heynei. Lupeol, a triterpene isolated from the plant, has been shown to possess cytotoxic activity against cancer cells [13]. The ethanolic extract of R. heynei has been found to exhibit significant wound healing activity in rats [14]. The plant's potential as an anticancer agent has also been evaluated in vitro and in vivo, with promising results [15]. In addition, Rhynchosiaheynei extracts possess various pharmacological activities, such as anti-inflammatory, analgesic, and antioxidant activities [16]. However, limited information is available regarding the antibacterial and antifungal activities of Rhynchosiaheynei extracts.

In this study, we investigated the antibacterial and antifungal effects of Rhynchosiaheynei whole plant extracts using zone inhibition method against four bacterial strains, including, *B. subtilis, S. aureus, P. aeruginosa, E. coli* as well as two fungal strains, Candida albicans and Aspergillus Niger.

Materials and Methods

Collection of plant material

In this work, R.heynei was the plant of choice originating from the Tirumala, India bioreserve. A voucher specimen of the plant (Accession No. 0791) was collected and validated by Dr. K Madhav Chetty of the Department of Botany at SV University, Tirupati.

Preparation of extract extraction

The powdered plant material (500 g) was subjected to extraction using hexane, diethyl ether, ethanol, and distilled water by the Soxhlet extraction method. After removing the impurities, the solvents were evaporated at low temperatures in a rotary evaporator. Extracts were dried and kept at 4 degrees centigrade until needed.

Test organisms and growth media

Four bacterial strains, namely Gram-positive Staphylococcus aureus (ATCC 25923) and Bacillus subtilis (ATCC 6633), and Gram-negative Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853), and two fungal strains, Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16404), were used in this study. All microbial strains were obtained from the American Type Culture Collection (ATCC). Bacterial strains were cultured on Mueller-Hinton agar (MHA), and fungal strains were cultured on Sabouraud dextrose agar (SDA) at 37°C and 28°C, respectively.

Antimicrobial activity by zone inhibition method

The antimicrobial activity of the four solvent extracts of R. heynei was evaluated using the Zone Inhibition Test on MHA for bacteria and SDA for fungi [18]. In brief, Sterile filter paper discs (6 mm diameter) were impregnated with different concentrations loaded (125, 250, 500, and 1000 µg/disk for bacteria and 125 to 5000 µg/disk for fungi) and were placed on the agar plates previously inoculated with test microorganisms (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, and Aspergillus niger). Ciprofloxacin (10 µg/disk) and fluconazole (25 µg/disk) were used as positive controls for bacteria and fungi, respectively. The plates were kept in an incubator at 37 degrees Celsius for 24 hours to grow bacteria and at 30 degrees Celsius for 48 hours to grow yeast. The diameter of the inhibition zone around each disc was measured in millimeters (mm) and recorded as the mean of three replicates.

Statistical analysis

The tests were run three times to ensure accuracy, and the data was presented as means +/- standard deviation (SD).

Results

Antimicrobial activity of *R. heynei*

Table 1 and 2 represent the inhibition zones diameter (IZD) of R. heyeni extracts (hexane, diethyl ether, ethanol and aqueous) against tested bacteria and fungi compared to ciprofloxacin and fluconazole,respectively.Gram-positive bacteria, B. cereus and S. aureus and Gram-negative bacteria, P. aeruginosa and E. coli were employed in the study to assess antibacterial property of R. heynei extracts. C. albicans and A. niger are the fungal species employed to assess antifungal activity. The IZD values were found upregulated in a concentration dependent manner in all the bacterial except in the case of E. coli, where the IZD was not concentration dependent.Ethanolic extract among all exhibited potent antimicrobial activity against S. aureus with an inhibition zone of 15 ± 1.41 mm at 500 µg/disk concentration. Similarly, Diethyl extract exhibited its highest antibacterial activity against S. aureus with an inhibition zone of 13.5 ± 0.70 mm at 1000 µg/disk concentration.While the hexane extract displayed highest IZD value 11 ± 0.00 mm at concentration 1000 µg/disk against P. aeruginosa. Aqueous extract of R. heynei failed exhibit antibacterial activity against B. subtilis and P. aeruginosa (except at a concentration of 1000 µg/disk). However, exhibited an inhibition zone

of 11 ± 0.00 mm against S. aureus. The positive control employed for antibacterial activity was ciprofloxacin which displayed a zone inhibition in range 27.5 ± 0.70 to 40 ± 0.00 mm and none of the extracts were able to achieve this range of inhibition. Similarly, there was concentration dependent increase in IZD against fungus C. albicans and A. niger. Diethylether and ethanol extracts exhibited highest IZD 10 ± 0.00 mm against A. niger at 5000 µg/disk concentration. However, hexane and aqueous extracts failed to display antifungal activity against A. niger, albeit they showed antifungal activity against C. albicans. As in the case of antibacterial activity, none of the extracts were capable to exhibit substantial antifungal activity like the positive control fluconazole which displayed an IZD ranging between 16 ± 2.82 to 37.5 ± 2.12 mm at 750 µg/disk concentration.

Table 1: Antibacterial activity of *R. heynei* extracts expressed as inhibition zone diameters (mm). PC* is ciprofloxacin given at 10µg/disk. Inhibition zone diameters are expressed as Mean ± SD

Microorganism	Concentration (µg/disk)	Hexane	Diethyl ether	Ethanol	Aqueous
B. subtilis	125	7 ± 0.00	7 ± 0.00	6 ± 0.00	0 ± 0.00
	250	7 ± 0.00	7 ± 0.00	7 ± 0.00	0 ± 0.00
	500	7 ± 0.00	6.5 ± 0.70	7 ± 0.00	0 ± 0.00
	1000	7.5 ± 0.70	7 ± 0.00	8 ± 0.00	0 ± 0.00
	2000	8 ± 0.00	8 ± 0.00	8.5 ± 0.70	0 ± 0.00
	PC*	29 ± 1.41	29.5 ± 0.70	28.5 ± 0.70	29 ± 0.70
S. aureus	50	7.5 ± 0.70	12.5 ± 0.70	10.5 ± 0.70	6 ± 0.00
	125	8.5 ± 0.70	11.5 ± 0.70	12.5 ± 2.12	9.5 ± 0.70
	250	10.5 ± 0.70	11.5 ± 0.70	11.5 ± 0.70	10 ± 0.00
	500	10 ± 0.70	12 ± 0.00	15 ± 1.41	10 ± 0.00
	1000	10.5 ± 0.70	13.5 ± 0.70	14 ± 0.00	11 ± 0.00
	PC*	29.5 ± 0.70	34.5 ± 0.70	34 ± 1.41	33 ± 0.00
P. aeruginosa	50	9 ± 0.00	10 ± 0.00	7.5 ± 0.70	0 ± 0.00
	125	10 ± 0.00	10 ± 0.00	7.5 ± 0.70	0 ± 0.00
	250	10.5 ± 0.70	10 ± 0.00	8 ± 0	0 ± 0.00
	500	10.5 ± 0.70	10.5 ± 0.70	8 ± 0	0 ± 0.00
	1000	11 ± 0.00	11 ± 0.00	7 ± 0	7 ± 0.00
	PC*	28.5 ± 0.70	27.5 ± 0.70	28 ± 0.00	28.5 ± 0.70
E. coli	125	9 ± 1.41	6.5 ± 0.70	9 ± 2.82	9 ± 1.41
	250	9.5 ± 3.53	7.5 ± 0.70	8 ± 0.00	7.5 ± 0.70
	500	8 ± 0.00	9 ± 2.82	7.5 ± 2.12	6.5 ± 0.70
	1000	7 ± 0.00	11.5 ± 0.70	6 ± 0.00	7 ± 1.41
	2000	7 ± 1.41	12 ± 0.00	6.5 ± 0.70	8.5 ± 2.12
	PC*	40 ± 0.00	38 ± 0.00	37.5 ± 2.12	38 ± 0.00

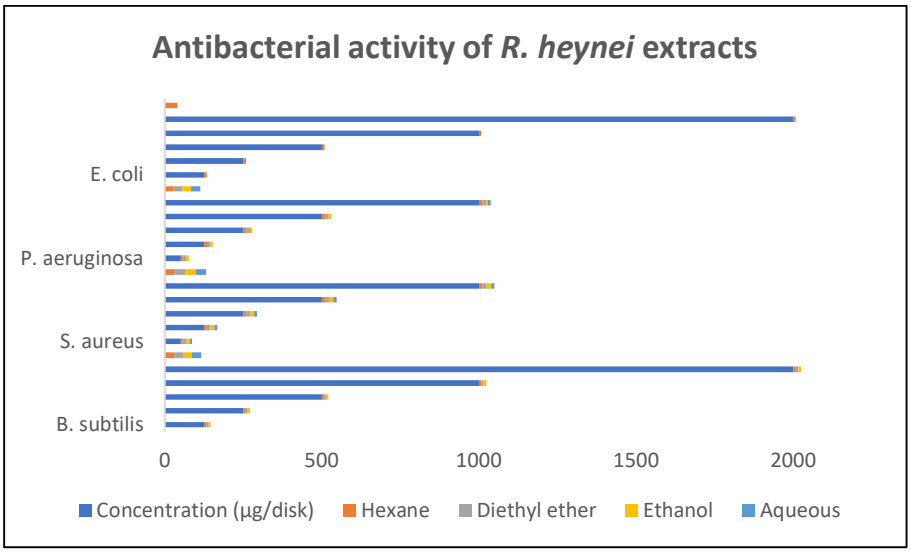
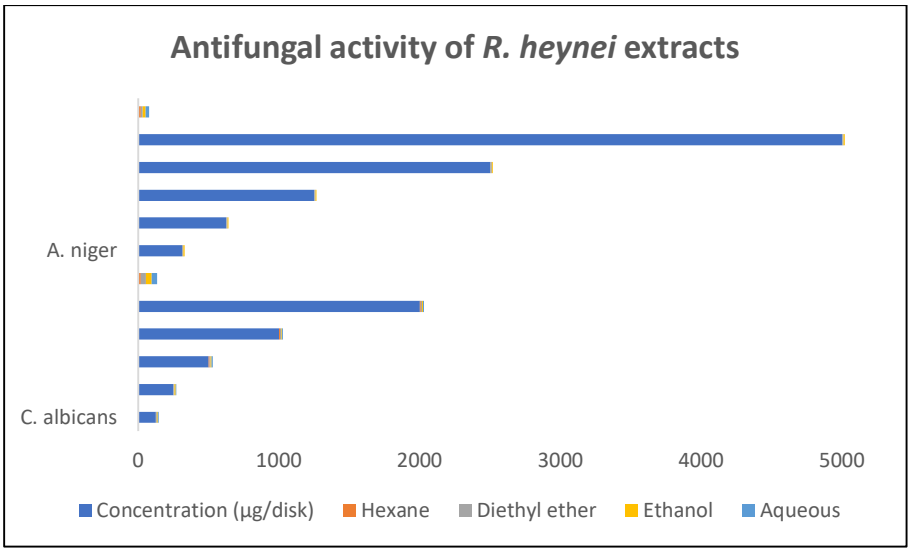


Figure 1: Antibacterial activity of *R. heynei* extracts expressed as inhibition zone diameters (mm)

Table 2: Antifungal activity of *R. heynei* extracts expressed as inhibition zones diameters (mm). PC# is Fluconazole given at 250µg/disk. Inhibition zone diameters are expressed as Mean ± SD

Microorganism	Concentration (µg/disk)	Hexane	Diethyl ether	Ethanol	Aqueous
C. albicans	125	0 ± 0.00	7 ± 0.00	6 ± 0.00	7.5 ± 0.70
	250	0 ± 0.00	6.5 ± 0.70	7.5 ± 0.70	7 ± 0.00
	500	6.5 ± 0.70	7 ± 0.00	8 ± 0.00	8.5 ± 0.70
	1000	7 ± 0.00	8 ± 0.00	7 ± 1.41	6.5 ± 0.70
	2000	7 ± 0.00	9 ± 0.00	7 ± 0.00	7.5 ± 0.70
	PC#	18 ± 0.00	38 ± 0.00	40 ± 0.00	37.5 ± 2.12
A. niger	312.5	0 ± 0.00	8.5 ± 0.70	7 ± 0.00	0 ± 0.00
	625	0 ± 0.00	8.5 ± 0.70	8 ± 0.00	0 ± 0.00
	1250	0 ± 0.00	9 ± 0.00	8 ± 0.00	0 ± 0.00
	2500	0 ± 0.00	9.5 ± 0.70	9.5 ± 0.70	0 ± 0.00
	5000	0 ± 0.00	10 ± 0.00	10 ± 0.00	0 ± 0.00
	PC#	16.5 ± 3.53	16 ± 2.82	18.5 ± 2.12	26 ± 0.00

Figure 2 Antifungal activity of R. heynei extracts expressed as inhibition zones diameters (mm)



Discussion

Rhynchosia species spread out in a large area throughout the world's tropical and subtropical regions [18]. Here and there, a few plants belonging to this genus employed in herbal remedies to treat a variety of illnesses, including antibacterial, antidiabetic, abortifacients, wound healing, hepatoprotective agents, and the treatment of boils, rheumatic aches, and skin infections [19–24]. Some identified compounds from the *Rhynchosia* genus and plant extracts have noteworthy antioxidant, anti-inflammatory, and other biological effects, antimycobacterial, and antiproliferative properties [25]. Previous phytochemical studies on *Rhynchosia* species revealed that the genus is solely responsible for the abundant synthesis of C-glycosylfavonoids. R. hevnei is one of the most popular herbs used for medicinal purposes among plants belonging to the *Rhynchosia* genus. Further, it is also widely evident that the search is on for potent antimicrobial agents that are naturally based, which can serve as substitutes for chemically synthesised medications that are potentially less toxic and effective against resistant microorganisms. The purpose of this study was to examine R. heynei's antibacterial potential in comparison to ciprofloxacin and fluconazole against the bacteria and fungi examined. The present study revealed that whole plant extracts of R. heynei exhibited a broad spectrum of antimicrobial activity. Different extracts showed varying degrees of antibacterial activity against all of the bacteria and fungus tested. The screening revealed that the Gram-positive bacteria, S. aureus, and Gram-negative bacteria, E. coli, were more susceptible to all the extracts. S. aureus was the most highly sensitive microorganism among others towards all four extracts, followed by E. coli, P. aeruginosa, and B. subtilis. Ethanol extract exhibited the highest IZD value, 15 ± 1.41 mm against S. aureus at 500 µg/disk concentration, which was half the IZD value exhibited by the positive control with 34 ± 1.41 mm at 10 µg/disk concentration. The next highest antibacterial activity was exhibited by diethyl ether extract with an IZD of 13.5 ± 0.70 mm against S. aureus. While the hexane extract was most active against P. aeruginosa with an IZD of 11 ± 0.00 mm. This activity of the extracts exhibited in the present study against S. aureus and P. aeruginosa was consistent with previous reports [26]. On the contrary, the aqueous extract could exhibit better antibacterial properties

against E. coli and S. aureus.Additionally, the assessment against fungi, C. albicans and A. niger, also revealed that R. heynei possessed decent antifungal properties. Between the two fungi employed, C. albicans was more sensitive to all the extracts. However, the highest IZD value exhibited against C. albicans (9 ± 0.00 mm by diethyl ether extract) was less than that observed with A. niger (10 ± 0.00 mm by diethyl ether and ethanol extract). Though hexane and aqueous extracts were inactive against A. niger, indicating it was not sensitive towards them. Both the fungi employed in the study were more susceptible to the antifungal property of diethyl ether extract. The variations in the antimicrobial property exhibited by these extracts could be due to the presence of different phytochemicals in different solvent extractions. According to the previous report, it was suggested that the antimicrobial activity was due to the existence of oxygenated monoterpenes, and these could be abundant in the ethanol and diethyl ether extractions of R. heynei. Further, characterization of each extract for the specific phytochemicals and their assessment is essential to understanding the underlying mechanism.

Conclusion

Extracts of R. heynei show considerable antibacterial action against the bacterial and fungal species examined, with the effectiveness increasing with increasing concentration. Among the different extracts, the ethanolic and diethyl ether extracts exhibited the highest antibacterial activity against S. aureus. The hexane extract showed the highest activity against P. aeruginosa, while the aqueous extract exhibited limited antibacterial activity. In terms of antifungal activity, the diethyl ether and ethanol extracts were found to be the most effective against A. niger, whereas the hexane and aqueous extracts did not show activity against A. niger but were active against C. albicans. However, the extracts did not show substantial antimicrobial activity compared to the positive controls, ciprofloxacin, and fluconazole, indicating the need for further investigation on isolating and characterizing the antibacterial constituents.

Acknowledgments

The authors are thankful to the Department of Pharmacognosy, KGRL College of Pharmacy, Bhimavaram, Andhra Pradesh, India and A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India for providing necessary facilities for carrying out the research work.

References

1. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-582.
2. Conlon JM, Sonnevend A, Davidson C, Nielsen PF, Alford RA. Antimicrobial peptides from ranid frogs: taxonomic and phylogenetic markers and a potential source of new therapeutic agents. BiochimBiophys Acta. 2004;1696(1):1-14.
3. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: a comparative overview. Evid Based Complement Alternat Med. 2005;2(4):465-473.
4. Walsh C. Molecular mechanisms that confer antibacterial drug resistance. Nature. 2000;406(6797):775-781.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012;75(3):311-335.

6. Sivapalan SR, Senthilkumar R, Kavimani S. Traditional medicinal plants used by the tribals of Kolli Hills, Tamil Nadu, India. J Nat Remedies. 2008;8(2):200-205.
7. Christapher SSR,Sivasankari SSSS, Felix T. Phytochemical screening and antioxidant activity of Rhynchosiaheynei Wight &Arn. Asian Pacific JTrop Biomed. 2012; 2(4):269-272.
8. Ross JA and Kasum JM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annual Rev Nutri. 2002; 22:19-34.
9. Ahmed FA andEl-Sayed AA. Phytochemical and pharmacological studies on the genus Rhynchosia L. (Fabaceae). J Appl Pharm Sci. 2017; 7(12): 156-165.
10. Nabavi SM, Nabavi SF,Ebrahimzadeh R, et al., Antioxidant and anti-cancer properties of the flavonoid quercetin. Curr Med Chem. 2014; 21(13): 1771-1777.
11. Gupta P, Bhatia PK, and Goyal MK. Phytochemical and pharmacological potential of tannins – a review. Food Chem Tox. 2015; 78:109-126.
12. Ravikumar KR, Paulsamy ND,Dhanaraj K, et al., Pharmacological evaluation of Rhynchosiaheynei Wight &Arn. for anti-inflammatory and analgesic activities. International Journal of Pharmaceutical Sciences and Research. 2014: 5(12): 5247-5252.
13. Prasad KM,Hussain BM, and Rao VC. Cytotoxic activity of lupeol isolated from Rhynchosiaheynei Wight &Arn. JPharm Res. 2009; 2(3): 408-411.
14. Subramoniam AV, Evans PJ, and Pushpangadan KR. Effect of Rhynchosiaheynei Wight &Arn. on dermal wound healing in rats. J Ethnopharmacol. 1998; 59(2) 115-119.
15. Latha SA, Sivasankari SSSS, and Ramesh P. In vitro cytotoxicity and in vivo antitumor activity of Rhynchosiaheynei. J Ethnopharmacol. 2012; 139(2): 442-446.
16. Srikanth J, Ragupathi R, Ravichandran V. Evaluation of anti-inflammatory, analgesic and antioxidant activities of Rhynchosiaheynei Wight and Arn. J Tradit Complement Med. 2017;7(2):206-211.
17. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. J Adv Pharm Tech & Res. 2011; 2(2):104.
18. Willis JCJ, Ferns A. A dictionary of the flowering plants and ferns. 7th ed. London: Cambridge University Press; 1966.
19. Rammohan A, Vijaya Kumar N, Ramakrishna S, Shankar BG, Jayaveera KN. Structure elucidation and antioxidant activity of the phenolic compounds from Rhynchosiasuaveolens. J Chem Pharm Res. 2015;7(4): 228-233.
20. Mary J, Raghavendra N, Subrahmanyam CJ. Antidiabetic and antioxidant activity of Rhynchosiabeddomei baker. Int J Med Res Rev. 2014; 2(1): 1323-1332.
21. Riaz T, Abbasi MA, Hussain T, et al. In vitro assessment of fortification from oxidative stress by various fractions of Rhynchosia pseudo-cajan. Afr J Biotechnol. 2011; 10(30): 6597-6603.
22. Allen ON, Allen EK. The Leguminosae, a source book of characteristics, uses, and nodulation. Madison, Wisconsin: University of Wisconsin Press; 1981.
23. Kinjo J, Hirota M, Tada Y, et al. Antiproliferative constituents in the seeds of Rhynchosiavolubilis. J Nat Prod. 2001; 24(12): 1443-1445.

24. Rondo M. Phytochemical and biological studies on some South African plants used in traditional medicine for skin hyperpigmentation [dissertation]. Johannesburg: University of Johannesburg; 2017.
25. Rammohan A, Vijayakumar N, Jayaveera KN. Phytochemistry and pharmacological activities of the genus Rhynchosia: a comprehensive review. Pharmacogn Rev. 2020; 14(27): 1-15.
26. Bhakshu LM, Raju RVJPB. Chemical composition and in vitro antimicrobial activity of essential oil of Rhynchosiaheynei, an endemic medicinal plant from Eastern Ghats of India. J Plant BiochemBiotechnol. 2009; 18(2): 1067-1070.