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Research Article

Phytochemical screening and evaluation of antioxidant activity of methanolic extract of Kenyan *Hydnora abyssinica* A. Braun (Hydnoraceae)

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Abstract

Hydnora abyssinica is a leafless medicinal herb characterized by rhizome and flower only. The decoction of the rhizome has been used in Kenya to manage infections, cancer and removal of retained placenta. Plant secondary metabolites that exhibit antioxidant activity are well documented and include mainly the phenolics. The objective of this study was to carry out phytochemical screening and investigate the antioxidant activity of the methanol extract of *H. abyssinica* rhizome. The extract was prepared by maceration. Phytochemical screening was done by standard methods and *in vitro* antioxidant activity was evaluated using 1,1-diphenyl-2-picryhydrazyl (DPPH) scavenging assay. Phytochemical studies revealed presence of alkaloids and glycosides which are reported for the first time being characteristic of this plant while tannins, phenols, steroids, flavonoids, terpenoids and fatty acids confirms reports of other similar studies on the Sudanese *Hydnora abyssinica*. Saponins, volatile oils and coumarins were not present in both the rhizome and flowers. DPPH free radical scavenging activity of the rhizome methanolic extract showed potential antioxidant activity with IC_{50} values of 26.7 µg/ml compared with that of 29.3 µg/ml for L-ascorbic acid standard. Activity of the methanolic extract may be associated with the presence of a number of varied classes of phytochemicals. This study perhaps pave way for further studies to provide data that would validate its medicinal uses and focus on bioassay guided fractionation and isolation of active compounds from its extracts.

Key words: Radical scavenging activity, DPPH, Maceration, Phytochemical screening

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1. Introduction

Hydnora abyssinica A. (Hydnoraceae) is a leafless medicinal herb characterized by rhizome and flower only. The plant is indigenous to Africa with currently four other species of the genus Hydnora that have been recognized and these are *H. Africana* T.*, H. Abyssinica* A.*, H. esculanta* J.*, H. triceps* D*. and H. Sinandevu* B. [10]. *H. abyssinica* A. synonymously referred to as *H. johannis* B.N. and *H. solmsiana* D. Sudanese traditional medical practitioners use it for curing severe bacterial infections [10,15] while in East Africa it is used as anti-diarrheal medicine, traditional medical practitioners in Kenya use the root decoction as a cure for throat complaints, as an astringent in dysentery, for treatment of stomach ache and for removing retained placenta during child birth [7]. Other reports indicate that it is used for the treatment of diarrhea, amoebic dysentery, typhoid, anthrax, East Coast Fever and cancer [9, 11] and as human and animal food [8, 10].

Natural antioxidants are secondary metabolites obtained from animal, plant, mineral or microorganism sources and prevent oxidation or formation of reactive oxygen species, such as superoxide (O_2), Hydroxyl (OH) and peroxyl (OOH, ROO) radicals, which commonly cause oxidative stress. The reactive oxygen species play an important role related to the degenerative or pathological process of various diseases such as aging, cancer, coronary heart disease, Alzheimer's disease, neuro degenerative disorders, atherosclerosis, cataracts and inflammation [2]. This study evaluated the antioxidant and phytochemistry of *H. abyssinica* with a view of justifying its utilization in management of degenerative diseases.

2. Materials and Methods

The rhizome and flowers of *H. abyssinica* were collected in November, 2013 from Kiangombe forest in Embu county, Kenya. The plant was identified at the site of collection by local traditional medicine practitioners who also provided detailed information on folklore uses. The voucher specimen of the plant was authenticated at the Herbarium section of National Museums of Kenya where it was deposited and its duplicate was deposited at Mount Kenya University Herbarium in the School of Pharmacy with a specimen voucher number HAO-1-2013. The materials were air-dried, ground. Sequential maceration was done successfully using petroleum ether, dichloromethane, dichloromethane :methanol mixture (1:1) and methanol. The extract was filtered, reduced *in vacuo* and completely oven-dried at temperatures set at 40^{o}C. The dried samples were stored in a freezer at - 20^{o}C until further evaluation for antioxidant activity.

Phytochemical analysis

The powders of roots and leaves were tested for the presence of bioactive compounds using standard methods.[3, 5].

Quantitative DPPH free radical scavenging activity

A stable radical of 1,1-diphenyl -2-picryhydrazyl (3.94 mg) was dissolved in methanol (100 ml) to give 100 µm solutions. To 3 ml of the methanolic solutions of DPPH was added 0.5 ml of each of the methanolic extract with concentrations of 0-640 µg/ml. The set up was kept incubated for 30 minutes in the dark and at room temperature, absorbance

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(A) was measured in triplicates for each sample at 517 nm using a SHIMADZU Multispect-1501 spectrophotometer. The percentage of the radical scavenging activity (RSA) was calculated by the following equation:

$RSA\% = [A_{control} - A_{sample}]/A_{control} \times 100$ and the obtained data was analyzed using GraphPad Prism Version 5 software.

DPPH Solution (3 ml) plus methanol (0.5 ml) was used as a negative control

Methanol (3.5 ml) was used as a blank while L-Ascorbic acid at concentrations equivalent to that of the test samples (0-640 µg/ml) was used as positive control as well as a standard reference [12,17].

3. Results and Discussion

Phytochemical analysis

The results of phytochemical tests, as shown in Table 1 indicated that the rhizome and flowers of *H. abyssinica* contain alkaloids, glycosides, tannins, phenols, steroids, flavonoids, terpenoids and fatty acids while saponins, volatile oils and coumarins were not present in both rhizome and flowers. The phytochemical screening results of this Kenyan *Hydnora abyssinica* are somehow consistent with the results of Sudanese *Hydnora abyssinica* for the presence of phenols, flavonoids and tannins. In contrast with the previous report on the absence of glycosides, in this study glycosides and alkaloids were reported for the first time [15].

The presence of quite a number of classes of secondary metabolites indicated above may be predictive of the ethnomedicinal claims on this medicinal plant. These phyto- constituents may be responsible for many pharmacological actions of the plant. The alkaloids have been associated with medicinal uses for centuries and one of their common properties is cytotoxicity but also analgesic, antispasmodic and antibacterial activities have been reported [13]. The glycosides are known to lower blood pressure and also especially cardiac glycosides have been used for over two centuries as stimulants in case of cardiac failure [13]. The anthraquinone glycosides have also been used as laxatives [13]. Steroids have antibacterial properties and they are also very important compounds especially due to their relationship with compounds such as sex hormones [13].

The phenolic compounds in this case flavonoids and tannins, possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-arteriosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [19]. This study of evaluation of antioxidant activity and phytochemical screening would perhaps lay a basis for further investigations based on the traditional uses of the plant in management of cancer and infectious diseases [13, 19].

Quantitative assay (*In vitro* DPPH free radical scavenging assay)

The 1, 1-diphenyl -2-picryhydrazyl (DPPH) radical scavenging activity of *H. abyssinica* rhizome methanolic (HA-MeoH) extract compared with standard antioxidant, L-ascorbic acid (ASA-Std) showed potential antioxidant activity with IC_{50} values of the extract and standard reference being 26.7 µg/ml and 29.3 µg/ml respectively.

The study also reveals that *H. abyssinica* methanolic extract exhibited concentration dependent scavenging activity by inhibiting the DPPH radicals generated. Similar studies on the Sudanese

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H. abyssinica ethanolic extract indicate that it has antioxidant activity using superoxide radicals [6].

Phytochemicals/Test	**Part of the plant**	
	Rhizome	**Flowers**
Alkaloids		
1. Mayer,s	+	+
2. Drangedorff	+	+
Cardioglycosides (Keller-killan)	+	+
Anthraquinone glycosides		
3. Borntragers	+	+
4. Modified Borntragers	+	+
Saponins	-	-
Tannins	+	+
Phenols	+	+
Steroids	+	+
Coumarins	-	-
Flavonoids	+	+
Volatile oils	-	-
Fatty acids	+	+
Terpenoids	+	+

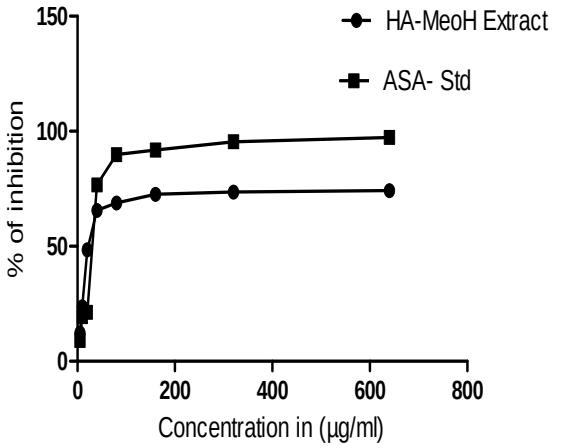
Table 1: Phytochemical tests of various parts of *Hydnora abyssinica*

4. Conclusion and Recommendation

In the light of the above results, phytochemical analysis results are important in the authentication of plant and further processing for isolation of active compounds from the plant. It can be predicted that the antioxidant activity may be due to the presence of phenolics. The findings that this plant has many a wide range of phytochemicals indicate that it may be explored for principles that may supply lead molecules. Keeping in view of this work, then suggestions for carrying out further studies on bioassay guided isolation especially using the and methanolic extracts to obtain and characterize potential antioxidant compounds may be warranted.

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Graph 1: In vitro antioxidant Assay (DPPH Assay)



IC_{50} HA-MeoH Extract-26.7

IC_{50} ASA-Std 29.3

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