Short Communication

Radical Scavenging Activity and Preliminary Phytochemical Screening of Pods of *Cassia arereh* Del. (Fabaceae)

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ABSTRACT

Cassia arereh is traditionally used as a fish poison and to manage different disease conditions including gastrointestinal tract (GIT) disorders, infertility, diabetes, insect bite, and infections. Free radicals have been implicated in the pathogenesis of a range of chronic diseases; and many medicinal plants are thought to be effective in managing such diseases, mainly through their free radicals scavenging ability. The objective of this study was to conduct phytochemical screening and investigate the free radical scavenging activity of various extracts of pods of *C. arereh***.** Petroleum ether, ethanol, and water extracts were prepared from the pods powder and tested for their radical scavenging activity using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay. The pods powder was also subjected to preliminary phytochemical screening. The results revealed that ethanol and water extracts possessed strong DPPH radical scavenging activity with 50% inhibitory concentration (IC_{50}) values of 8.84 and 16.76µg/ml, respectively. Ascorbic acid was used as a standard and exhibited a radical scavenging IC_{50} value of 2.0µg/ml. Results of preliminary phytochemical screening indicated the possible presence of anthraquinones, carbohydrates, deoxy-sugars, saponins, tannins, and terpenoids. It can be concluded that pods of *C. arereh* may contain medicinally relevant constituents such as terpenoids and displayed strong radical scavenging activity, which may partly contribute to the possible scientific basis for its traditional use to alleviate different disease conditions.

Keywords: *Cassia arereh*, Phytochemical screening, Radical scavenging, Traditional medicine, Ethiopia.

1. INTRODUCTION

Cassia arereh is a woody, wild small tree widely spread in India and tropical Africa (Olusola et al., 2011). It is distributed in some regions of Ethiopia such as Tigray, Gondar, Gojam, Kefa and Shewa uplands; and it is known by different local names: *Shitol hibey* (Tigrigna), *Kerkay* (Agew). It is a deciduous woody plant often occurring in rocky places or riparian habitats. It is traditionally used as fish poison and to alleviate different disease conditions including gastrointestinal tract (GIT) disorders, infertility, diabetes, insect bite, and a range of infections (Hedberg and Edwards, 1989; Yineger et al., 2008; Etuk et al., 2010; Gibree et al., 2013; Mustapha, 2013). The plant is reported to show different biological activities such as antimicrobial, *in vitro* antitrypanosomial, larvicidal, antiplasmodial, and cytotoxic properties.

Reports claim that *C. arereh* contain alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins, and terpenoids (De et al., 2009; Ngulde et al., 2010; Olusola et al., 2011; Akanbi and Nnakaogu, 2012; Imam et al., 2013; Ngulde et al., 2013; Ado et al., 2014). Many studies have shown that natural antioxidants can reduce Dioxide ribonucleic acid (DNA) damage, mutagenesis, carcinogenesis, and inhibit growth of pathogenic bacteria. These events are often associated with the termination of free radical propagation in biological systems. Accordingly, the antioxidant capacity is widely used as a parameter for evaluating medicinal bioactive components (Ko et al., 2009; Gulçin et al., 2011). Hence, the objective of this study was to carry out phytochemical screening and free radical scavenging activity investigation on various extracts of pods of *C. arereh***.**

2. METHODOLOGY

2.1. Methods

2.1.1. Collection

Pods of *C. arereh* were collected in April 2013 from Kolla Tembien, Central Zone of Tigray in northern Ethiopia. The plant material was authenticated in the National Herbarium, Addis Ababa University, Ethiopia.

2.1.2. Extraction

Dried pods were opened, seeds were removed, and dirty matters were carefully cleaned. Deseeded and cleaned pods were then cut in to small pieces manually and then powdered using grinder. To make activity testing, different solvent extracts were prepared employing Soxhlet and reflux extraction methods. Hundred gm of the powdered pods of *C. arereh* was packed in a thimble and subjected to successive Soxhlet extraction using petroleum ether and ethanol, each time for 24 hours. While the petroleum ether and ethanol extracts were concentrated and dried, the remaining marc was refluxed with distilled water three times, each time for one hour, because the high boiling point of water does not make it convenient solvent for soxhlet extraction. The water extracts were combined, filtered, concentrated under reduced pressure using Rota Vapor and dried in a vacuum oven at a temperature of 35^{o}C. In all cases, the dried extracts were transferred into vials and stored for further use.

2.1.3. Preliminary Phytochemical Screening

Preliminary phytochemical screening of pods of *C. arereh* was conducted following the procedures described by Debella, (2002) with slight modifications.

2.1.4. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Assay

The procedure described by Sokmen et al. (2005) has been followed with slight modification to measure the DPPH radical scavenging activity of different extracts of pods of *C. arereh* and ascorbic acid (a reference compound). Fifty µl of various concentrations (ranging from 100 to 400µg/ml) of test samples were added to 5ml of 0.004% MeOH solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read using UV/Vis spectrophotometer (Jenway, Model 6305) against a blank (methanol) at 517nm. Percent inhibition of the free radical DPPH was calculated according to the formula: % Inhibition = $[(A_c - A_s)/A_c] \times 100$; where, A_c is the absorbance of the control (0.004% MeOH solution of DPPH without test sample) and A_s is the absorbance of the test sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as percentage inhibition against test sample concentration. Tests were carried out in triplicates.

3. RESULTS

3.1. Extraction

The three solvents (petroleum ether, ethanol, and water) used for extraction produced extracts of different percentage yields (w/w) as summarized in table 1. Ethanol extract had the highest percentage yield (12.35%) followed by aqueous extract (7.4%) and petroleum ether (0.5%). Similarly, the three extracts displayed varied organoleptic properties: petroleum ether extract was yellowish, ethanol extract was greenish, and water extract was reddish brown; but after drying, all had a reddish brown color with semisolid consistency. The ethanol and aqueous extracts displayed chocolate like odor with the former having more pronounced odor.

3.2. Preliminary Phytochemical Screening

Phytochemical screening of pods of *C. arereh* indicated the possible presence of anthraquinones, carbohydrates, deoxy-sugars, saponins, tannins, and terpenoids; but alkaloids, flavonoids, phenols and steroids were not detected (Table 2).

Table 1**.** Characteristic Color and Consistency of Different Extracts of Pod of *C. arereh*.

Type of Extract	**Characteristic Color and Consistency**		**Percentage yield (w/w)**
	Before drying	**After drying**	
Petroleum Ether	Yellowish, less viscous liquid	Reddish brown semisolid	0.50
Ethanol	Greenish, viscous liquid	Reddish brown semisolid	12.35
Distilled Water	Reddish brown, viscous liquid	Reddish brown semisolid	7.40

Table 2**.** Summary of Results on Phytochemical Screening of Pod of *C. arerh*.

Phytochemical group	**Test**	**Results**
Carbohydrates	Fehling's A test	+
	Benedict's test	+
	Molisch's test	+
Alkaloids	Dragendroff's test	-
	Mayer's test	-
Deoxy sugar	Keller-kilani test	+
Steroids	Salkowski's test	-
Phenols	Ferric Chloride test	-
Tannins	Gelatin test	+
Flavonoids	Shinoda test	-
Saponins	Froth test	+
Anthraquinones	Borntrager's test	+

(***Key***: + = detected, - = not detected).

Table 3. DPPH Radical Scavenging IC_{50} Values of Different Extracts of Pod of *Cassia arereh*.

Type of Extract	**IC_{50} Values (µg/ml)**
Petroleum Ether	113.12
Ethanol	8.84
Distilled Water	16.76

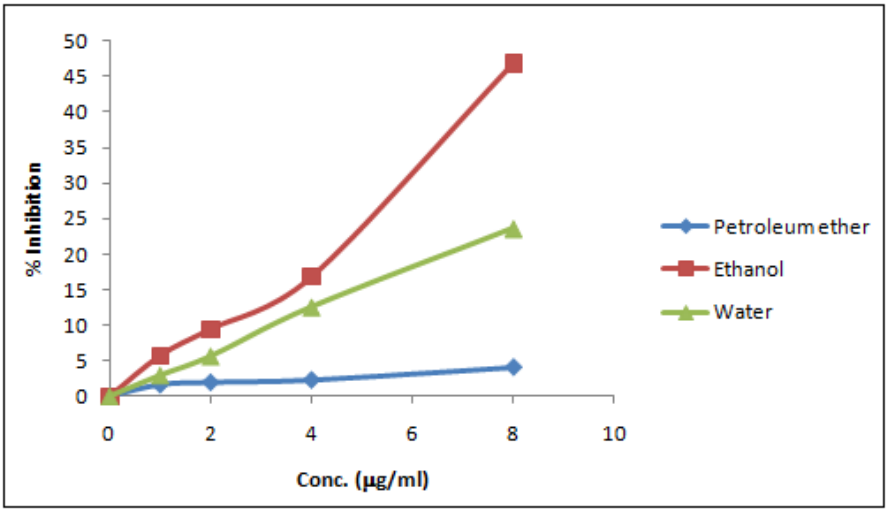

Figure 1. Radical Scavenging Activities of Different Extracts of pods of *C. arereh*.

3.3. Free Radical Scavenging Activity

As shown in figure 1, all the extracts demonstrated concentration dependent DPPH radical scavenging activity; and concentrations at which the test samples decreased the amount of

DPPH radical by 50% (IC_{50} values) were calculated from the graph plotted as percentage inhibition against test sample concentration. IC_{50} values were used to compare the radical scavenging activity of extracts; and ethanol, water, and petroleum ether extracts displayed IC_{50} values of 8.84, 16.76, and 113.12 μg/ml, respectively (Table 3).

4. DISCUSSION

4.1. Extraction

Different extraction solvents of varied polarity were employed in the present study to identify an appropriate extraction solvent that can produce an extract from pod of C. *arereh* with higher yield or potent activity. The relatively less percentage yield of the petroleum ether fraction (Table 1) could indicate that pods of *C. arereh* contained less fatty or waxy substances; and the highest yield of ethanol extract indicate that alcoholic or hydro alcoholic solvents could be appropriate extraction solvents to get an extract of higher yield from the pods. After drying, the ethanol and water extracts had chocolate like characteristic color and odor with the former having more pronounced odor. These organoleptic properties could be considered as important physical properties, especially the characteristic odor, that can be utilized during quality control of this plant because it could be potential source of raw material to the agricultural and/or pharmaceutical industries as food supplement, pharmaceutical additives or alternative herbal remedy.

4.2. Preliminary Phytochemical Screening

As shown in table 2, anthraquinones, carbohydrates, deoxy-sugars, saponins, tannins, and terpenoids were detected in the extracts of the pods, whereas alkaloids, flavonoids, phenols and steroids were not detected. Other phytochemical studies on different parts of this plant reported the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins (phlobatannins), and terpenoids (De et al., 2009; Ngulde et al., 2010; Olusola et al., 2011; Akanbi and Nnakaogu, 2012; Imam et al., 2013; Salihu and Ado, 2013). These reports support the present work and it can be suggested that anthraquinones, carbohydrates, saponins, tannins, terpenoids, and possibly cardiac glycosides (positive Keller-kilani test) could be constituents of the pods of *C. arereh*; but alkaloids, flavonoids, phenols and steroids were not detected indicating that these constituents may be absent or may not exist in an appreciable amount in the pods although these constituents can be detected in other part of the plant such as root, bark, and leaf.

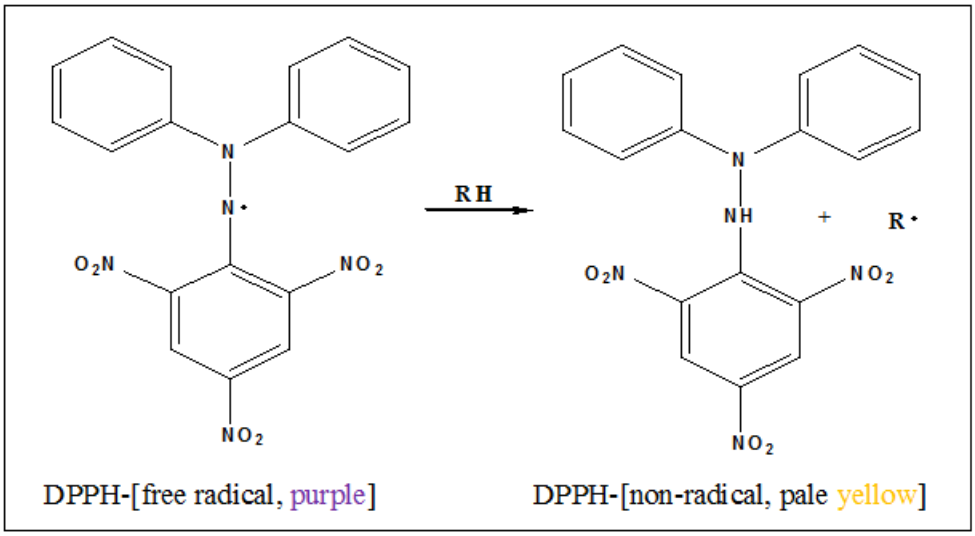

Figure 2. Structural Illustration of Reaction between DPPH and Free Radical Scavengers.

4.3. Free Radical Scavenging Activity

DPPH is a stable free radical which bears a deep purple color (absorbs at 517 nm). This assay is based on measurement of the reducing ability of antioxidants toward DPPH-radical (Prior et al., 2005). Upon reduction, the color of the solution fades and the reaction progress is conveniently monitored by a spectrophotometer (Huang et al., 2005) (Fig 2). This assay measures the free radical scavenging activity of test samples. When measured accordingly, the ethanol and aqueous extracts of pods of *C. arereh* displayed promising radical scavenging activities. A number of reports use IC_{50} values (concentrations at which test samples decrease the amount of DPPH radical by 50%) to compare the radical scavenging activity strengths of different extracts. For example, Kukic et al. (2006) considered $IC_{50} < 50$ µg/ml as high radical scavenging activity and Hajdu et al. (2007) considered IC_{50} of 3643 µg/ml as less radical scavenging activity. In the present study, ethanol, water, and petroleum ether extracts displayed IC_{50} values of 8.84, 16.76, and 113.12, respectively (Table 3), is showing that the ethanol extract displayed the highest DPPH radical scavenging activity. Ascorbic acid, well known antioxidant that was used as a standard, had a radical scavenging IC_{50} value of 2µg/ml. So far, this is the first report on antioxidant or radical scavenging activity of pods of *C. arereh;* and based on the results, it can be claimed that ethanol and aqueous extracts of pods of *C. arereh* demonstrated strong radical scavenging activity whereas the petroleum ether extract showed moderate activity.

5. CONCLUSION AND RECOMMENDATION

The ethanol and aqueous extracts of pods of *C. arereh* exhibited strong DPPH radical scavenging activities; and contained medicinally relevant phytoconstituents such as terpenoids. Ethanol extract had the highest yield and radical scavenging activities compared to petroleum ether and aqueous extracts. The extracts possessed chocolate like characteristic odor which could be taken as vital organoleptic evaluation parameter while considering quality control of the pod of this plant. Because pods of *C. arereh* could be a potential source of raw material for agricultural and pharmaceutical industries as food supplement, pharmaceutical additives and alternative herbal remedy, more study is recommended to further verify and exploit its potential.

6. ACKNOWLEDGEMENTS

The authors are grateful to Mr. Asfaw Hailu of the National Herbarium, Addis Ababa University (AAU) for authentication of the plant material; Course and Research Unit of Pharmacognosy, College of Health Science, Mekelle University for providing facility and material support.

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