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Research Article

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Assessment of Total Content of Secondary Metabolites, *In Vitro* Free Radical Scavenging Potential and Peroxide Value on Different Solvent Extracts of *Rumex abyssinicus* Jacq. Root

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories. These are primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contains like terpenoids, flavonoids, saponins, tannins, alkaloids, glycosides and phenols. Secondary metabolites or phytochemicals are byproducts of plants that are important for the survival of it in their immediate environment. But these chemicals do have medicinal value to humans. The present study drawn in qualitative identification of secondary metabolites from the medicinal plant roots of Rumex abysinicus Jacq. available around Debre Tabor town. The extraction was carried out by four different solvents; such as methanol, ethyl acetate, petroleum ether and chloroform. These crude extracts of the roots of the plant were used for the phytochemical analysis to find out the secondary metabolites constituents and both peroxide value and DPPH determination were used for antioxidant activity evaluation. The results confirmed the presence of secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, saponins, tannins, cartenoids, phlobatannins, steroids and glycosides depending on the type of solvent used for extraction. Peroxide value and DPPH assay methods also showed antioxidant activities of root extracts of based on Rumex abysinicus Jacq. Methanolic extract showed lowest peroxide value (PV) (51 meq/kg) and highest percentage of inhibition (86%I) and the highest PV were recorded in ethyl acetate extracts (101 meq/kg). 20.3%I was the lowest value recorded in methanol extracts for DPPH assay.

Keywords: Phytochemicals; DPPH; Peroxide value; Antioxidant; *Rumex abysinicus* Jacq.

INTRODUCTION

Since time immemorial, mankind has used plant extracts from different plants to cure many diseases and thus relieve him from physical agony [1]. Medicinal plant is an important element of indigenous medical systems in all over the world [2,3]. Nearly 80% of the world populations rely on traditional medicines for primary health care, most of which involve the use of plant extracts [3]. The medicinal properties of these traditional plants are due to phytochemicals constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Important phytochemicals include alkaloids, flavonoids, anthocyanins, tannins, terpenes, phenolics, vitamins etc. [4]. Ethiopia is endowed with a diverse biological resources including about 6, 500 species of higher plants, with approximately 12% endemic, hence

making it one of the six plant biodiversity rich regions [5]. *Rumex abysinicus* Jacq. is one of the medicinal plants found in Ethiopia, it is is a large annual herb up to 4 m high. Its local Amharic name is 'Mekmako'. In Ethiopia, it has been traditionally used for management of hypertension, inflammatory and painful conditions traditionally Peoples use the root powders of *Rumex abysinicus* Jacq. as sweating agent for tea, and also they use the powders of the root as a coloring for butter production [6]. But scientifically there were no enough studied about the use of different parts of the plant extracts and also qualitative and quantitative phytochemical investigations were not carried out. Therefore the objective of the research was to determination of the antioxidant activity of root of *Rumex abysinicus* Jacq. using peroxide value and DPPH free radical scavenging activities and investigate the phytochemicals present in the sample qualitatively and quantitatively in methanol, chloroform, petroleum ether and ethyl acetate solvents.

MATERIAL AND METHODS

Plant Material

The roots of *Rumex abyssinicus* Jacq. were collected from countryside near to Debre Tabor town, after collecting the root of the plant, it was washed properly with distilled water and cut into small portions and then placed at room temperature (23°C) without sun light to be dried. The dried roots were powdered by electrical grinder and stored in a clean polyethylene bag until extraction was carried out.

Extraction of Plant Material

20 gram powdered roots of *Rumex abyssinicus* Jacq. ware added to 200 ml methanol, ethyl acetate, petroleum ether and chloroform in a separate conical flask and shacked for 48 hours. Each solution was filtered by using Whatman number 1 filter paper in a separate conical flask and concentrated in rotary evaporator at 35°C.

Preliminary Phytochemical Screening

The roots of *Rumex abyssinicus* Jacq. extract was used for preliminary screening of phytochemicals such as phenols [7], Flavonoids [8], Tannins [8], Alkaloids [9], Saponins [9], Terpenoids [10], Steroid [10], Glycosides [11], Quinine [11], Phlobatannin and Carotenoids [12].

Quantitative Phytochemical Determination

Determination of total alkaloid: A total of 200 ml of 20% acetic acid was added to 5 gram root powders of *Rumex abyssinicus* Jacq. in a separate 250 ml beaker and covered to stand for 4 h. The mixture was filtered and the volume was reduced to one quarter (1/4) using water bath. To this sample concentrated ammonium hydroxide (NH_4OH) was added drop wise until the precipitate was completed. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [13].

Determination of total flavonoid: 10 g of the root of *Rumex abyssinicus* Jacq. was extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No.1. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and the evaporating dish was weighed to a constant weight [14].

Determination of total saponin: A measured weight 5 g of the powdered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55°C; it was then filtered through Whatman filter paper no.1. The residue was extracted with 50 ml of 20 ethanol

and both extract were poured together and the combined extract was reduced to about 40 ml at 90°C and transferred to a separating funnel and 40 ml of diethyl ether was added and shaken vigorously. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponnins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a reweighed evaporating dish. It was dried at 60°C in the oven and reweighed after cooling [15].

Peroxide value determination: The peroxide value of Niger seed oil was determination using Iodometric titration method techniques [16]. Five different samples that contains Niger seed oil and methanol extracts of roots of *Rumex abyssinicus* Jacq., Niger seed oil and ethyl acetate extracts of roots of *Rumex abyssinicus* Jacq, Niger seed oil and chloroform extracts of roots of *Rumex abyssinicus* Jacq, Niger seed oil and petroleum ether extracts of roots of *Rumex abyssinicus* Jacq. and Niger seed oil only for control were prepared in 250 ml Erlenmeyer flask. The samples were placed at room temperature (23°C). From each sample 5 gram were taken and 30 ml of acetic acid and chloroform (3:2) v/v solution were added to each sample. The flask was shaken until the sample was completely dissolved. Then 0.5 ml of saturated KI Solution was added to the flask, which was then stoppered and the contents of the flask were shaken for exactly 1 min. 30 ml of distilled water was added and shaken vigorously to librates iodine from chloroform layer. 1 ml of 1% starch solution was added into the flask as an indicator, and the mixture was titrated with 0.1 N sodium thiosulfate solution until the blue gray color disappeared in the aqueous phase. Three replicates were performed per each sample. A blank determination was performed under the same condition. These experiments were done four times with four day intervals. All these experiments were carried out at room temperature (23°C).

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the root extract was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) [9]. 1 ml of 0.1 mM DPPH was added to 1 ml of methanol, chloroform, ethyl acetate and petroleum ether root extract of *Rumex abysinicus* Jacq. with concentrations (50, 125, 250, 500, and 1000) µg/ml. The mixtures were left to stand for 30 min in the dark and the absorbance was recorded at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and higher absorbance shows lower free radical scavenging activities [17]. An equal amount of DPPH and Methanol served as control. The experiment was done in triplicate. Ascorbic acid was used as standard control. The percentage scavenging was calculated using the following formula,

DPPH Scavenging effect (%)=[(AC - AS / AC) x 100]

Where: AC absorbance of the control and AA absorbance of sample.

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation.

RESULTS AND DISCUSSION

The amounts of crude extract from 20 gram root powder of the *Rumex abysinicus* Jacq. were shown in Figure 1.

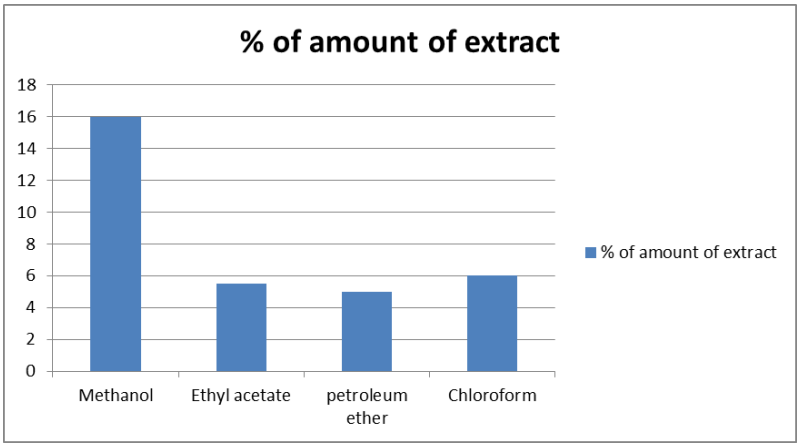

Figure 1. Percentage of crude extracts of roots of *Rumex abysinicus* Jacq. using different solvent

Methanol extracts showed highest amount of crude extracts (16.2%) compared to the amount obtained using ethyl acetate (5.7%), petroleum ether (5%) and chloroform (6%) solvents. The lowest amount was recorded in chloroform extracts. This indicated that the polarity of solvent influences the extraction of crude product from the root of *Rumex abysinicus* Jacq. According to the graph the percentage of crude extraction was decreased from methanol to chloroform, ethyl acetate and petroleum ether.

Phytochemical Analyses

For testing the presence of selected secondary metabolites in root extracts *Rumex abssinicus Jacq*., different secondary metabolites test were carried out and results were recorded (Table 1).

Table 1. Phytochemical screening of secondary methabolities from roots of *Rumex abysinicus Jacq*

	Types of solvents			
Secondary metabolites	**Methanol**	**Chloroform**	**Ethyl acetate**	**Petroleum ether**
Phenols	+	-	-	-
Flavonoids	+	-	-	-
Terpenoids	-	+	+	+
Tannins	+	-	-	-
Alkaloids	-	+	+	+
Glycosides	+	+	-	-
Steroids	-	-	+	+
Saponnins	-	+	+	+
Phlobatannins	-	-	-	-
Carotenoids	-	-	-	-

+: Present; -: Absent

The result confirmed that the extract of root of *Rumex abysinicus* Jacq. has different secondary metabolites. The polarity of the solvent used for extraction influences the presence and amount of the secondary metabolites present on it. Highly polar solvents such as methanol extracts polar secondary metabolites while less polar solvents such as petroleum ether, ethyl acetate and chloroform extract less polar or non-polar secondary metabolites. From the above result phenols, flavonoids, tannins and glycosides were extracted by highly polar solvent methanol. Terpenoids, alkaloids and saponins were extracted by less polar solvents such as petroleum ether, ethyl acetate and chloroform. Steroids were extracted by very less polar solvents petroleum ether and ethyl acetate.

Total Secondary Metabolites Determination

From root powder of *Rumex abssinicus* Jacq. the percentage of alkaloid, flavonoid and Saponin were determined and the result was showed in Figure 2.

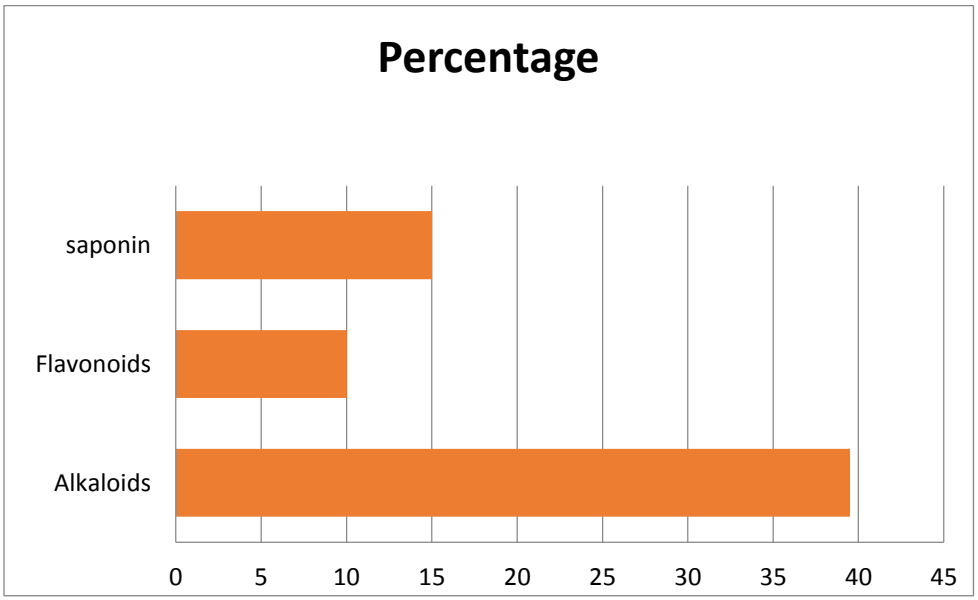

Figure 2. Total content of alkaloid, saponin and flavonoid in percentage

The percentage of alkaloid is the highest as compared from flavonoid and saponin. This implies that the root of *Rumex abysinicus* Jacq. has the highest alkaloid content. A little amount of flavonoid and saponin also present in roots of *Rumex abysinicus Jacq.*

Peroxide Value Determination

Peroxide value is a widely measure of the primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation [18]. Peroxide value of control (Niger seed oil bought from market) was 107 meq/kg for the first treatment. The peroxide value of control after 16 days was increased to 193 meq/kg. These change of peroxide value significantly indicated the noticeable phenomenon of lipid oxidation. This showed that free radicals were generated during lipid oxidation when increasing days of storage this is due to increments of the peroxide value. Peroxide value of methanol crude extract containing Niger seed oil was 51 meq/kg for the first treatment. But the peroxide values increase to 115 meq/kg at the completion of treatment (after 2 weeks). Peroxide value of chloroform crude extract containing Niger seed oil was changed from 69 meq/kg to 139 meq/kg and peroxide value of niger seed oil that contain petroleum ether extracted root plants also changed from 95 meq/kg to 175 meq/kg. Peroxide values of ethyl acetate containing Niger seed oil also changed from 101 meq/kg to 185 meq/kg from first treatment to final treatment.

In each treatment PV of Niger seed oil containing methanol crude extract, chloroform crude extract, petroleum crude extract and ethyl acetate crude extract was increased. The peroxide value increments might be due to the increase of primary products (peroxides) followed by an increase in secondary products (aldehydes and ketones) [19]. The peroxide value of control was significantly higher than the peroxide value of all other treatments. The peroxide value of ethyl acetate crude extract containing Niger seed oil was higher than the peroxide value of petroleum ether, chloroform and methanol crude extracts containing Niger seed oil. Treatment containing methanol crude extract was significantly has lower peroxide value than all other treatments. This showed that ethyl acetate and petroleum ether had the lowest antioxidant activity whereas methanol crude extract had the highest antioxidant activity than the other treatment.

DPPH Free Radical Scavenging Determination

The free radical scavenging activities of *Rumex abisinicus* Jacq. were studied by its ability to reduce the DPPH. In DPPH assay, the methanol, chloroform, ethyl acetate and petroleum ether root extract of the plant showed antioxidant activity. However at highest concentration the methanolic extracts showed highest value (90.4 ± 0.04% l) and petroleum ether showed the lowest value (42.8 ± 0.07% l). DPPH scavenging activity was ranging from 86 ± 0.07% to 90.4±0.04% in the case of methanol root extract, whereas in the case of petroleum ether extracts ranging

(20.3 ± 0.67% I) to (42.8 ± 0.007% I). In all extract, as concentration increase scavenging activity also increase. In methanol, chloroform, ethyl acetate and petroleum ether extract, the highest percentage of inhibition were 90.8%I, 73.4%I , 66%I , 42.8%I and the lowest were found 86%I, 51.2%I, 43.2%I, 20.3%I respectively. The reduction in the number of DPPH molecule can be correlated with the available number of hydroxyl groups. Hence the significant scavenging activity may be due to the presence of hydroxyl groups present in phytochemicals of the root of *Rumex abysinicus* Jacq. (Figures 3 and 4) [20].

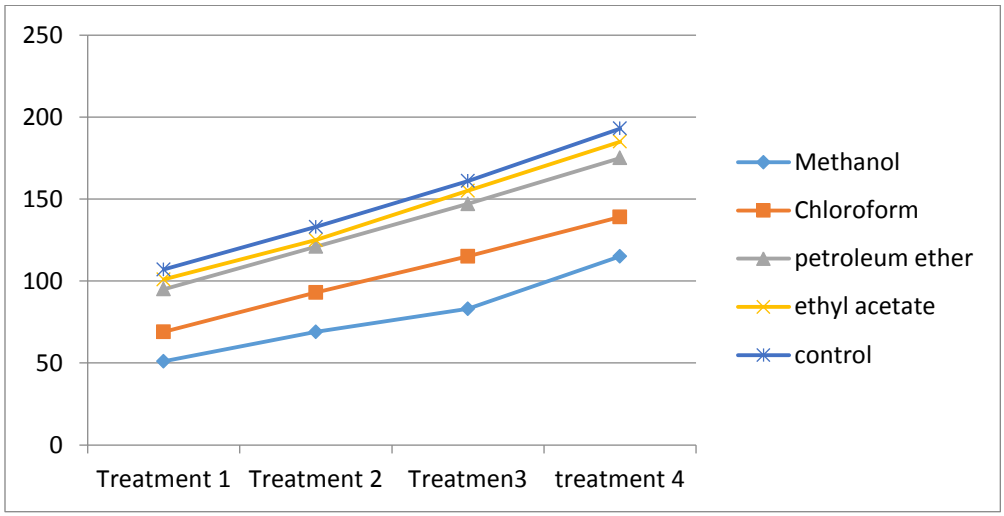

Figure 3. Peroxide value of different solvent extraction of roots of *Rumex abisinicus Jacq*.

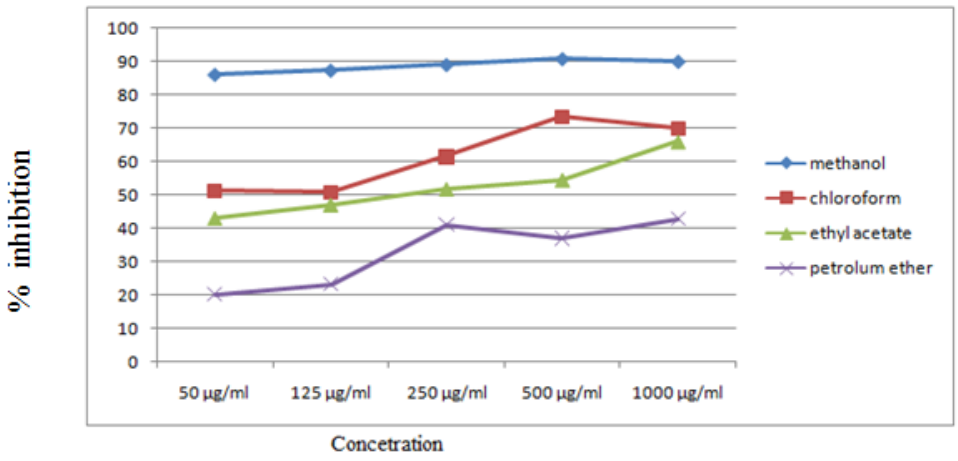

Figure 4. Percentage inhibition of extract of roots *Rumex abisinicus* Jacq. on DPPH

CONCLUSION

Above mentioned premilarly secondary metabolites test reveal that root of *Rumex abisinicus* Jacq. has different secondary metabolites. Methanol has the highest ability to extract secondary metabolites from the roots of *Rumex abisinicus* Jacq. as compared from the other three solvents. Phytochemicals extracted from roots of *Rumex abisinicus* Jacq. showed good antioxidant activity for lipid containing in niger seed oil and DPPH free radical scavenging potential. A methanol crude extract root has high antioxidant activity. Petroleum ether crude extract and ethyl acetate crude extract had low antioxidant activity. The antioxidant activities of the extracts due to the presence of secondary metabolites listed in Table 1 because secondary methabolities have special antioxidant properties [21]. Peoples can use the root extracts of *Rumex abisinicus* Jacq. to prevent themselves from the effect of free radicals.

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