Journal of The Faculty of Science and Technology (JFST)
http://journal.oiu.edu.sd/index.php/JFST
https://doi.org/10.52981/jfst.vi8.1958
ISSN: 1858 - 6007
مجلة العلوم و التقانة – جامعة أم درمان الإسلامية – وزارة التعليم العالي و البحث العلمي - السودان

Research article **JFST Issue No. 8 (2021) 24 – 29**

Phytochemical Analysis of *Rheum officinale* Rhizomes marketed in Central Sudan

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Abstract: The purpose of this study is to extract and analyze the athraquinone from Rhubarb (*Rheum officinale*) rhizomes by chromatographic techniques (silica gel and polyamide plates) and to determine anthraquinone quantitatively using UV spectrophotometer. Thin layer chromatography (TLC) and Polymide plates were performed to confirm the presence of phenolic compounds. Solvent system, Toluene: Ethyl acetate: Formic acid (5:4:1) achieved good separation for petroleum ether fraction (7spots), chloroform fraction (4 spots), ethyl acetate (4 spots) whereas crude ethanolic extracts showing 2 spots after spraying with vanillin sulphuric acid whereas solvent system, petroleum ether: ethyl acetate (5:3) achieved separation for ethyl acetate fraction showing 2spots on gray light after spraying with aluminum chloride. The petroleum ether extract was read in UV spectrophotometer and give result equal 2.578 which considered the total content of free anthraquinone in the plant rhizome.

Keywords: Phytochemical analysis, *Rheum officinale*, Central Sudan.

Introduction

Rhubarb is the common name of the root of several different species of *Rheum.* the parts of the plant used medicinally include both the root and the rhizome. Root has a purgative action for use In the treatment of constipation but also has an astringent effect too. it, therefore, has a truly cleansing action upon the gut, removing debris and then astringing with antiseptic properties as well (USDA, 2009; Bisset, 1994). The primary chemical constituents of Rhubarb include anthraquinones, chrysophanol , emodin

,physeion ,sennidine , rheidine , palmmidie , tannins , catechin , gallic acid ,oxalic acid , rutin , phytosterol and calcium oxalate .it is anthraquinones that contribute to the laxative and purgative properties of rhubarb ,yet the tannin content helps balance those properties , and even stops diarrhea . Rhizome used for treatment of occasional constipation and hypotension , increase peripral vasodilation , and inhibit blood coagulation (Yizhong et al., 2004; Bradley, 1992; Nishioka , 1991; Goding, 1976).

The major constituents are hydroxyanthracene derivatives (2-5%) including emodin , physcione aloe-emodin , and chrysophanol glycosides ,along with di-O , C-glucosides of the monomeric reduced from (rheinosides A-D) and dimeric reduced forms (sennosides A-F) the level of oxidized forms is maximal in summer and almost nil in the winter (Peigen et al., 1984).

Until the 1950, chrysophanol and other anthraquinones were considered to be the constituents producing the purgative action of rhubarb .current evidence indicates that the major active principle are the dimeric sennosides A-D . It also contains (gallotannins) , flavonoid (2-3%) and naphtohydroquinone glyconside (Bruneton, 1995); Nishioka , 1991).

The objective of this study is to extract and analyze anthraquione and phenolic compouds from *Rheum officiale* rhizomes then using Thin Layer chromatography (silica gel and polymide plates) and to estimate the total content of free anthraquinone.

Experimental

Plant material

The sample of plant rhizomes was purchased from Omdurman market , dried and grinded to make plant root powder .

Extraction

50gm of root powder were placed in 500ml conical flask , and then 300ml 70 % ethanol were added . The conical flask was placed in the water bath and was allowed to stand for 1 hour , the time was measured after boiling start (after appearance of the first bubble) . The mixture (powder and ethanol) was filtered using filter paper while it is hot by using another 500ml conical flask , 2ml of the filtered extract were placed in a vial labeled as crude extract .

Heating was continued until the smell of ethanol disappeared. The whole concentrated extract was placed in separating funnel and was fractionated with 30ml petroleum ether shacked gently (3time) . Two layers were formed . The petroleum ether layer placed in porcelain dish and heated in water bath to concentrate it. Then placed in a vail

labeled as “petroleum ether fraction”. Remaining aqueous layer was placed again in the separating funnel and was fractionated by The 25ml of chloroform were added and shacked gently (3times) . Two layer were formed, the chloroform layer (lower one) was placed in porcelain dish and heated in water path to concentrated it . Then 2ml transferred into a vail labeled as” chloroform fraction “

The remaining aqueous extract was placed again in the separating funnel , and 21ml of ethyl acetate were added and shacked gently until two layer were formed (3 times) the ethyl acetate layer was placed in a porcelain dish and concentrated in water bath , then 2ml were taken into avail labeled as “ ethyl acetate fraction “ then , 2ml of water residue were taken and placed in avail labeled as “ water residue fraction “ .

Thin layer chromatography:

Chromatographic analysis was applied to the above fraction by using thin layer chromatographic method “ using silica gel F254 and polyamide plate “ . 12 ml of each solvent system (petroleum ether: ethyl acetate (5:3)). (toluene ethyl acetate: formic acid (5:4:1)) were placed in different clean dry beakers , and then each beakers covered with foil and petri dish and allowed to saturated 4 different spot of petroleum ether , ethyl acetate ,chloroform and crude extract fraction were applied using 4 different capillary tube to silica gel plate at the baseline .which measured as 0.5cm from the bottom . 1cm from the left and right side the plate was put in beaker contain solvent system (toluene: ethyl acetate: formic acid (5:4:1) for the polyamide plate spot of ethyl acetate, curd extract and water residue were applied to the plate at the baseline , using different capillary tube . then put beaker contain (ethanol : water (5:3)) solvent system . the two plate were allowed to run for 75% of the plate . then the plate were taken out and the solvent front were determined and then allowed to dry at room temperature .

The spot were seen in the daylight and under UV in both short wavelength 254 and long wavelength 365 . then silica gel plate it was sprayed using vanillin sulphuric acid and put in oven at 100 C for 1minuet and seen in the daylight . The polyamide plate was sprayed with natural product reagent and seen under U.V lamb in both short and long length.

Estimation of total content of free anthraquinone

100ml of the defatted powder of Rhubarb root was refluxed for 20minute with 2ml of 10%(W/V) FeCl, 2ml of 10 MHCl was added and the mixture was refluxed for a further 20minute . The cooled mixture was extracted with petroleum ether and the extract was evaporated to dryness . the residue was treated with 10ml of 5% NaOH containing 2%

of NH_3 and after 20minute the absorbance of the solution was measured at 520nm using UV spectrophotometer (double beam) (Elujoba *et al* ., 1989) .

Results and Discussion:

Thin layer chromatography (TLC) and polyamide plate were performed to confirm the presence of phenolic compounds in *Rheum officiale* rhizomes. For TLC. violet and yellow colour spots were identified when observed in day light after sprayed with vanillin sulphuric acid . For polyamide plate , orange color spots were identified when viewed in day light after sprayed with aluminum chloride.

From Table 1 it is evident that solvent system : toluene : ethyl acetate : formic acid (5:4:1) achieved good separation for petroleum ether fraction (7spots), chloroform fraction (4 spots) , ethyl acetate (4 spots) whereas crude ethanolic extracts showing 2 spots after spraying with vanillin sulphuric acid. From Table 2 it evident that solvent system , petroleum ether : ethyl acetate (5:3) achieved separation for ethyl acetate fraction showing 2spots on gray light after spraying with aluminum chloride.

The petroleum ether extract was read in UV spectrophotometer (double beam) and give result equal 2.578 which considered the total content of free anthraquinone in the plant .

Table 1. The result of TLC examination of phenolic in *R.officinale* using solvent system , toluene : ethyl acetate : formic acid (5:4:1) and spray reagent vanillin sulphuric acid

Extract	**Observation in day light**	**R_f value**
Petroleum ether	Faint violet	0.05
	Faint violet	0.52
	violet	0.8
	yellow	0.82
	violet	0.87
	yellow	0.91
	violet	0.97
Chloroform	Yellow	0.22
	Yellow	0.78
	Yellow	0.88
	Violet	0.97
Ethyl acetate	Yellow	0.18
	Yellow	0.81
	Yellow	0.92
	Violet	0.97
Crude extract	Faint yellow	0.2
	Faint yellow	0.92

Table 2. The result of polyamide examination of phenolics in *R.officinale* using solvent system , petroleum ether : ethyl acetate (5:3) and spray reagent aluminum chloride :

Extract	**Observation in day light**	**R_f value**
Ethyl acetate	Orange	0.17
	Orange	0.31
Crude extract	Faint orange	0.16
Water residue	Faint orange	0.15

Conclusion

The anthraquinones and phenolic compounds were extracted with ethanol 70% and was fractionated with three solvents (petroleum ether ,chloroform , and ethyl acetate). The ethanolic extract in addition to the fraction were applied to separation by TLC and polyamide plates. The total content of the free anthraquinones in *Rheum officinale* rhizome was estimated using UV spectrophotometer (double beam) .

Therefore, it is recommended that more has to be carried out concerning the identification of the isolated compounds by using different analytical methods including IR, H1 and C13 NMR.

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